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Proposed Registration Decision

Beauveria bassiana strain GHA

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Overview

Proposed Registration Decision for *Beauveria bassiana* strain GHA

Health Canada's Pest Management Regulatory Agency (PMRA), under the authority of the *Pest Control Products Act* and Regulations, is proposing full registration for the sale and use of *Beauveria bassiana* Technical, Botanigard ES and Botanigard 22 WP, containing the technical grade active ingredient *Beauveria bassiana* strain GHA, to control whitefly, aphids and thrips in greenhouse ornamentals and vegetables.

An evaluation of available scientific information found that, under the approved conditions of use, the product has value and does not present an unacceptable risk to human health or the environment.

This Overview describes the key points of the evaluation, while the Science Evaluation provides detailed technical information on the human health, environmental and value assessments of *Beauveria bassiana* Technical, Botanigard ES and Botanigard 22 WP.

What Does Health Canada Consider When Making a Registration Decision?

The key objective of the *Pest Control Products Act* is to prevent unacceptable risks to people and the environment from the use of pest control products. Health or environmental risk is considered acceptable[1] if there is reasonable certainty that no harm to human health, future generations or the environment will result from use or exposure to the product under its proposed conditions of registration. The Act also requires that products have value[2] when used according to the label directions. Conditions of registration may include special precautionary measures on the product label to further reduce risk.

To reach its decisions, the PMRA applies modern, rigorous risk-assessment methods and policies. These methods consider the unique characteristics of sensitive subpopulations in humans (e.g. children) as well as organisms in the environment (e.g. those most sensitive to environmental contaminants). These methods and policies also consider the nature of the effects observed and the uncertainties when predicting the impact of pesticides. For more information on how the PMRA regulates pesticides, the assessment process and risk-reduction programs, please visit the PMRA's website at www.healthcanada.gc.ca/pmra.

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1 "Acceptable risks" as defined by subsection 2(2) of the *Pest Control Products Act*.

2 "Value" as defined by subsection 2(1) of the *Pest Control Products Act*: "the product's actual or potential contribution to pest management, taking into account its conditions or proposed conditions of registration, and includes the product's (a) efficacy; (b) effect on host organisms in connection with which it is intended to be used; and (c) health, safety and environmental benefits and social and economic impact."

Before making a final registration decision on *Beauveria bassiana* strain GHA, the PMRA will consider all comments received from the public in response to this consultation document[3]. The PMRA will then publish a Registration Decision[4] on *Beauveria bassiana* strain GHA, which will include the decision, the reasons for it, a summary of comments received on the proposed final registration decision and the PMRA's response to these comments.

For more details on the information presented in this Overview, please refer to the Science Evaluation of this consultation document.

What Is *Beauveria bassiana* strain GHA?

Beauveria bassiana strain GHA is a microbial pest control agent in the end-use products Botanigard ES and Botanigard 22 WP. These end-use products provide control of whitefly, aphids and thrips on greenhouse ornamentals and vegetables.

Beauveria bassiana is a fungus that grows naturally in soils throughout the world and acts as a parasite on various insect species. *B. bassiana* is a "generalist entomopathogenic fungi", which is a fungus that causes a disease in many types of insects. In the case of *B. bassiana,* it causes a disease called "white muscardine disease" in most insects. While insects living in or near the soil have evolved natural defences against this fungus as it is common in their natural environment, it can be used as a biological insecticide against most other insects.

Health Considerations

Can Approved Uses of *Beauveria bassiana* strain GHA Affect Human Health?

Beauveria bassiana strain GHA is unlikely to affect human health when used according to the label directions

People could be exposed to *B. bassiana* strain GHA when handling and applying the products. The PMRA considers several key factors when assessing health risks: the microorganism's biological properties (e.g. production of toxic byproducts), reports of any adverse incidents, its potential for pathogenicity, infectivity and toxicity as determined in toxicological studies as well as the likely levels to which people may be exposed to this strain relative to exposures already encountered in nature to other strains of this microorganism.

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3 "Consultation statement" as required by subsection 28(2) of the *Pest Control Products Act*.

4 "Decision statement" as required by subsection 28(5) of the *Pest Control Products Act*.

Toxicological studies in laboratory animals describe potential health effects from large doses in hopes of identifying any potential pathogenicity, infectivity and toxicity concerns. *Beauveria bassiana* Technical was found to be a mild eye irritant; therefore, the product labels will have the appropriate label statements.

No other significant toxicity or signs of disease were observed when *B. bassiana* strain GHA was tested on laboratory animals.

Residues in Water and Food

Dietary risks from food and water are not of concern

The *Food and Drugs Act* prohibits the sale of food containing a pesticide residue that exceeds the established maximum residue limits (MRL). Pesticide MRLs are established for *Food and Drugs Act* purposes through the evaluation of scientific data under the *Pest Control Products Act*. Each MRL value determines the maximum concentration in parts per million (ppm) of a pesticide allowed in or on certain foods. Food containing a pesticide residue that does not exceed the established MRL does not pose an unacceptable health risk.

Strains of *B. bassiana* are common in nature and the use of Botanigard ES and Botanigard 22 WP in greenhouses is not expected to significantly increase natural environmental background levels of this microorganism. Furthermore, no significant adverse effects were reported when *B. bassiana* strain GHA was administered orally to rats. Therefore, the establishment of an MRL is not required for *B. bassiana* strain GHA. As well, the likelihood of residues contaminating drinking water supplies is negligible to non-existent. Consequently, dietary exposure and risks are minimal to non-existent.

Occupational Risks From Handling Botanigard ES and Botanigard 22 WP

Occupational risks are not of concern when Botanigard ES and Botanigard 22 WP are used according to label directions, which include protective measures.

Workers using Botanigard ES and Botanigard 22 WP can come into direct contact with *B. bassiana* strain GHA on the skin, in the eyes or by inhalation. For this reason, the label will specify that workers using Botanigard ES and Botanigard 22 WP must wear gloves, long-sleeved shirts, long pants, a NIOSH approved respirator (with any N, P, R or HE filter) and shoes plus socks. Eye goggles are also required when handling Botanigard 22 WP. Early-entry workers will also be restricted from entering areas where Botanigard ES and Botanigard 22 WP have been applied for a period of 12 hours unless wearing the indicated personal protective equipment.

For bystanders, exposure is expected to be much less than that of handlers and mixer/loaders and is considered negligible. Therefore, health risks to bystanders are not of concern.

Environmental Considerations

What Happens When *Beauveria bassiana* strain GHA Is Introduced Into the Environment?

Environmental risks are not of concern.

Information on the environmental fate of *B. bassiana* strain GHA suggests that, as a common soil microorganism, it is likely that *B. bassiana* strain GHA could survive in outdoor soil under favourable environmental conditions (i.e. moisture, acidity levels); however, the populations of *B. bassiana* strain GHA would return to natural background levels over time.

The effects of *B. bassiana* strain GHA on birds, earthworms, fish, aquatic arthropods, terrestrial and aquatic plants and beneficial and/or environmentally important insects were examined. Although the risk to the non-target organisms was found to be acceptable, to protect beneficial insects, precautionary statements regarding the safe use of Botanigard ES and Botanigard 22 WP in integrated pest management strategies will be required on end-use product labels, as will be specific instructions to minimize exposure to bees. To reduce the potential for phytotoxic effects on crop plants, advisory statements notifying users to spot test plant surfaces before applying product for the first time and to minimize the accumulation of visible residues on plant surfaces will also be required on the end-use product labels under the directions for use section.

Although avian pulmonary/inhalation/injection, wild mammal and microorganism testing was not conducted, adequate information was available to determine that significant adverse effects to these non-target organisms are not expected.

Value Considerations

What Is the Value of Botanigard ES and Botanigard 22 WP?

Botanigard ES and Botanigard 22 WP are reduced-risk biopesticides that can be used to control whiteflies, aphids and thrips on greenhouse vegetables and ornamentals.

These are biological control products that may be used on greenhouse ornamentals and vegetables in place of conventional chemical insecticides. The data reviewed demonstrated Botanigard ES and Botanigard 22 WP will generally provide acceptable control of whiteflies, aphids and thrips on greenhouse vegetables and ornamentals. However, depending on humidity and temperature, efficacy can be variable. Under unfavourable conditions, these products may not always provide good control. This is not unexpected given the mode of action of these products (a fungal biological control agent that causes a disease). In addition, these are lower risk products compared to older conventional insecticides with the same use pattern.

Measures to Minimize Risk

Labels of registered pesticide products include specific instructions for use. Directions include risk-reduction measures to protect human and environmental health. These directions must be followed by law.

The key risk-reduction measures being proposed on the labels of Botanigard ES and Botanigard 22 WP to address the potential risks identified in this assessment are as follows.

Key Risk-Reduction Measures

Human Health

Due to concerns with users developing allergic reactions through repeated high exposure to *B. bassiana* strain GHA, anyone handling or applying Botanigard ES or Botanigard 22 WP must wear waterproof gloves, a long-sleeved shirt, long pants and shoes plus socks. In addition, mixers/loaders and applicators must wear a dust/mist filtering mask. Furthermore, anyone handling or applying Botanigard 22 WP will be required to wear eye goggles as this formulation is a moderate eye irritant.

Environment

As a general precaution, the label prohibits the direct application of the products to aquatic habitats (such as lakes, rivers, sloughs, ponds, prairie potholes, creeks, marshes, streams, reservoirs and wetlands), estuaries or marine habitats. The label also directs handler to not contaminate surface water by disposal of equipment wash waters.

To protect beneficial insects, users will be instructed to minimize overspray in the greenhouse. Labelling will also warn users that Botanigard ES and Botanigard 22 WP may be harmful to beneficial insects and to avoid direct contact with beneficial insects.

As some strains of *B. bassiana* have been shown to be toxic to honeybees, users are directed to avoid applying the products to areas where honeybees are actively foraging.

To reduce the potential for phytotoxic effects on crop plants, advisory statements notifying users to spot test plant surfaces before applying product for the first time and to minimize the accumulation of visible residues on plant surfaces are required on the label under the directions for use section.

One of the formulants in Botanigard ES contains heavy aromatic petroleum distillates, which are toxic to aquatic organisms and will, therefore, be labelled as such on the end-use product label.

Next Steps

Before making a final registration decision on *Beauveria bassiana* Technical, Botanigard ES and Botanigard 22 WP, the Pest Management Regulatory Agency (PMRA) will consider all comments received from the public in response to this consultation document. The PMRA will accept written comments on this proposal up to 45 days from the date of publication of this document. Please forward all comments to Publications (contact information on the cover page of this document). The PMRA will then publish a Registration Decision, which will include its decision, the reasons for it, a summary of comments received on the proposed final decision and the Agency's response to these comments.

Other Information

When the PMRA makes its registration decision, it will publish a Registration Decision on *Beauveria bassiana* strain GHA (based on the Science Evaluation of this consultation document). In addition, the test data referenced in this consultation document will be available for public inspection, upon application, in the PMRA's Reading Room (located in Ottawa).

Science Evaluation

Beauvaria bassiana strain GHA

1.0 The Active Ingredient, Its Properties and Uses

1.1 Identity of the Active Ingredient

Active microorganism	*Beauveria bassiana* strain GHA
Function	Suppress whitefly, aphids, and thrips on ornamentals and vegetables in commercial greenhouses
Binomial name	*Beauveria bassiana* strain GHA
Taxonomic designation	
Kingdom	Eumycota
Phylum	Dikarymycota
Subphylum	Ascomycotina
Class	Pezizomycotina
SubClass	Sordariomycete
Order	Hypocreales
Family	Clavicipitaceae
Genus	*Beauveria*
Species	*bassiana*
Strain	GHA
Patent status information	No Canadian patent status information was provided.
Minimum purity of active	1.4×10^{14} conidia/kg

Identity of relevant impurities of toxicological, environmental and/or significance	The technical grade active ingredient does not contain any impurities or micro contaminants known to be TSMP Track 1 substances. Although it does not appear on the List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern (*Canada Gazette*, part II, Volume 139, Number 24, pages 2641–2643), a component of one of the formulants in Botanigard ES is considered to be toxic as defined in section 64 of the *Canadian Environmental Protection Act,* 1999. The PMRA has conducted a risk assessment of this formulant component and has found that the associated risk is acceptable for the proposed use. Beauvericin, a secondary metabolite of *B. bassiana* strain GHA, has been identified in the technical product. Each production lot will be monitored for the presence of this metabolite.

1.2 Physical and Chemical Properties of the Active Substances and End-use Product

Technical Product—*Beauveria bassiana* Technical

Property	Result
Colour	Off-white (near Pantone #12-0808)
Odour	Slightly musty
Physical state	Fine powder
Guarantee	1.34×10^{11} conidia/mL
Density	0.183 ± 0.12 g/cm^3
Storage stability	Dry place at -20°C
Flammability	N/A
Explodability	Non-explosive
pH	5.8

End Use Product—Botanigard ES

Property	Result
Colour	Yellow-brown (near Pantone #16-1333)
Odour	Petroleum based aromatic smell
Physical state	Turbid liquid
Formulation type	Emulsifiable suspension
Guarantee	11.3% *Beauveria bassiana* Technical
Specific gravity	0.93 g/mL
Storage stability	9 months when stored at 25°C
Corrosion characteristics	Non-corrosive
Flammability	N/A
Explodability	N/A
Flash point	182°C

End Use Product—Botanigard 22 WP

Property	Result
Colour	Gray (near Pantone #12-1006)
Odour	Slight petroleum odor
Physical state	Non-dusty, slightly cohesive powder
Formulation type	Wettable powder
Guarantee	22.0% *Beauveria bassiana* Technical
Bulk density	0.512 ± 0.003 g/cm^3
Storage stability	9 months when stored at 25°C
Corrosion characteristics	N/A
Flammability	N/A
Explodability	N/A
pH	6.3

1.3 Directions for Use

Crops:	Greenhouse ornamentals and vegetables
Pests:	Whiteflies, aphids, and thrips
Application Rates	**Botanigard ES** • Whiteflies and aphids: 0.5 to 1 L per 400 L spray (1.25 to 2.5 mL/L or 0.125% to 0.25%) • Thrips: 2 L per 400 L spray (5 mL/L or 0.5%) **Botanigard 22 WP** • Whiteflies and aphids: 250 g to 500 g per 400 L spray (0.625 to 1.25 g/L or 0.0625% to 0.125%) • Thrips: 500 g to 1 kg per 400 L spray (1.25 to 2.5 g/L or 0.125% to 0.25%)
Application Interval:	5– to 10–day intervals, 2– to 5–day intervals with high insect populations
Number of Applications:	Repeat for as long as pest pressure persists
Application Timing:	Begin treatment at first appearance of the pest, most effective when used early before high insect populations develop
Spray Volume:	Spray to wet, but avoid runoff
Pre-Harvest Interval:	0 days

1.4 Mode of Action

The mode of action of *Beauvaria bassiana* is as a generalist Entomopathogenic fungus, which causes a disease called white muscardine disease in insects. When spores of the fungus come into contact with the body of an insect host, they germinate, enter the body of the insect and grow hyphae, eventually killing the insect. After death, a white mould grows on the cadaver and produces new spores. Most insects living in or near the soil have evolved natural defences against this fungus as it is common in the natural environment.

2.0 Methods of Analysis

2.1 Methods for Identification of the Microorganism

Beauveria bassiana strain GHA is a *Hyphomycete* fungus, producing conidia on exposed conidiophores. The genus *Beauveria* is closely related to the genera *Tritirachium* and *Acrodontium*. *Beauveria bassiana* strain GHA can be identified to the species level by microscopic examination of morphological features, such as conidial production and ramification, by the method of deHoog (1972).

Strain specific identification of *B. bassiana* strain GHA is achieved by DNA fingerprinting methods (Restriction Fragment Length Polymorphisms analysis of chromosomal DNA) and allozyme analysis.

2.2 Method for Establishment of Purity of Seed Stock

A master stock of *B. bassiana* strain GHA is stored at -80°C, from which stock cultures are prepared and stored at -20°C on a yearly basis. Replicates are also stored at the American Type Culture Collection (ATCC) as ascension number ATCC 74250.

Practices for ensuring the purity of the seed stock were adequately described in the summary of the method of manufacture and quality assurance program.

2.3 Methods to Define the Content of the Microorganism in the Manufactured Material Used for the Production of Formulated Products

Total fungal spore counts in the technical product and both end-use products are taken using a hemacytometer.

The potency (viable conidia/g) of the technical grade active ingredient is measured by microscopic examination of germinated and ungerminated spores from plated dilutions of technical grade active ingredient. The potency of Botanigard ES is measured by microscopic examination of germinated versus ungerminated spores from plated dilutions of product with lactofuschin staining, while the potency of Botanigard 22 WP is measured directly from a diluted sample of the product. The product guarantee represents results from potency analyses.

2.4 Methods to Determine and Quantify Residues (Viable or Non-viable) of the Active Microorganism and Relevant Metabolites

Beauvericin is a secondary metabolite produced by *B. bassiana* strain GHA that has been isolated and identified in the technical grade active ingredient using an acceptable method. To ensure that levels of beauvericin in the technical product do not exceed acceptable levels, the registrant proposes to monitor all future production batches.

2.5 Methods for Determination of Relevant Impurities in the Manufactured Material

The quality assurance procedures used to limit contaminating microorganisms during manufacture of Botanigard ES and Botanigard 22 WP are acceptable.

Contamination in the liquid and solid preculture is monitored using plate counts on standard media following standard methods (incubation at 25°C and 37°C) to detect any unusual colonies and to verify colony morphology. Contamination is also evident in the solid pre-culture by unusual odours or colours, or zones of poor fungal growth. If contamination is detected in the liquid preculture, the liquid preculture is discarded. Contamination in the solid pre-culture is first characterized (i.e. level of contamination, competition with the desired fungus, stage of

growth/sporulation) and, in the event of gross fungal contamination, the batch of solid pre-culture is steam sterilized and discarded.

The technical product and both end-use products are monitored for contaminants using pathogen specific media. Gross contamination would also be distinguished during the microscopic examination of diluted suspensions, and plated diluted samples during potency and viability assessments. Release standards for microbial contaminants in the end-use products comply with those permitted by the PMRA and are adequate for detecting human and animal microorganisms of concern (see Section 2.6).

2.6 Methods to Show Absence of Any Human and Mammalian Pathogens

As noted in Section 2.5, microbe-specific screening methods for total bacteria and total fungi, total enteric bacteria, including *Salmonella* spp., *Shigella* spp., *Proteus* spp., *Escherichia coli*, *Pseudomonas* spp. and *Vibrio* spp are adequate for detecting and enumerating microbial contaminants of concern and to ensure that Botanigard ES and Botanigard 22 WP do not contain unacceptable levels of human and animal disease-causing microorganisms.

2.7 Methods to Determine Storage Stability, Shelf-life of the Microorganism

Results from storage stability testing from three lots each of Botanigard ES and Botanigard 22 WP at room temperature (25°C) showed that both end-use products are stable for a period of up to 9 months under these conditions. At 35°C, a decrease in viability was experienced after 1-3 months.

3.0 Impact on Human and Animal Health

3.1 Toxicicity and Infectivity Summary

A survey of published literature has revealed some cases of human infection in immunocompromised individuals from *Beauveria bassiana*. The cases include reports of deep tissue infection, pulmonary mycosis, empyema and corneal keratitis. Given the ubiquitous nature of *B. bassiana* as a common soil microorganism, cases of systemic infection are considered rare. *B. bassiana* does not generally infect healthy individuals and available antifungal therapies offer an effective treatment. Cases of corneal keratitis caused by *B. bassiana* have arisen following traumatic eye injury or surgery, but there has been excellent prognosis for complete cure through appropriate therapeutic treatment. Studies with *B. bassiana* indicate that, like most fungal species, *B. bassiana* has some allergic potential. A summary of work-related injuries from the manufacturing/formulating facility of Mycotech Corportation indicated that there were no work-related injuries/incidents reported in the year 2000–2001.

A detailed review of the toxicological database for *B. bassiana* strain GHA has been completed. The database for *B. bassiana* strain GHA is complete (see Appendix I), consisting of laboratory animal (in vivo) toxicity studies testing the technical grade active ingredient (acute oral toxicity/pathogenicity and infectivity, acute pulmonary toxicity/pathogenicity and infectivity and

acute intraperitoneal infectivity) and the end-use products (acute dermal irritation), which are currently required for health hazard assessment purposes. These studies were carried out in accordance with currently accepted international testing protocols and Good Laboratory Practices. Although the dermal toxicity study did not entirely comply with the guidelines, the information in the study was considered adequate to assess the potential for dermal toxicity. Therefore, a replacement study was not required.

In addition to the required studies, a dermal sensitzation study and a bacterial reverse mutation assay with *B. bassiana* Technical were also submitted. To support the safety of the end-use products, acute oral toxicity and eye irritation testing were conducted with formulations equivalent to Botanigard ES (Mycotrol ES9601, Mycotrol Botanigard ES) and Botanigard 22 WP (Mycotrol WP9611b, Mycotrol WP 91616b, Mycotrol Botanigard 22 WP). The scientific quality of the data is high and the database is considered sufficient to characterize the toxicity and infectivity of this pest control agent and associated end-use products.

In an acute oral toxicity and infectivity study, no mortalities, no significant toxicity and no necropsy findings were observed in CD® Rats following oral gavage with *B. bassiana* strain GHA in 0.1% Tween 80 at 1.03 × 108 CFU per animal. Test substance had cleared from all tissues/fluids by day three. The 7-day oral LD50 is > 1.0×10^8 CFU/animal (males and females). Based on the results of this study, *B. bassiana* strain GHA is of low toxicity, and is not pathogenic, in the CD Rat via the oral route.

In an acute oral toxicity study (limit test), Mycotrol ES9601 (1.21×10^{10} conidia/g) was of low toxicity in Sprague-Dawley rats following a single oral dose of undiluted product at 5 g/kg bw. The 14-day oral LD_{50} for rats is > 5 g/kg bw (male and female).

Two independent acute oral toxicity studies (limit tests) were conducted with Mycotrol WP9611b (4.52×10^{10} spores/g). In both studies, Mycotrol WP9611b was of low toxicity in Sprague-Dawley rats (Crl:CDRBR) following a single oral dose of 5 g/kg bw in a suspension of 50% corn oil. General signs of toxicity were observed in some animals from each study on the day of dosing, but all signs were resolved by day five or sooner with the exception of one male test animal that exhibited discoloration around the mouth, and abnormal breathing intermittently throughout the study. The 14-day oral LD_{50} is > 5 g/kg bw (males and females).

In an acute pulmonary toxicity and infectivity study, no mortalities were observed in CD Rats following intratracheal administration of *B. bassiana* strain GHA in 0.1% Tween 80 at 1.01 × 108 CFU per animal. A generalized inflammatory response occurred in animals treated with live and heat-killed test substance, but the inflammatory response was resolved by day 14. There was evidence of slight systemic toxicity in females dosed with viable test substance, but all rats returned to normal by day 14. Test substance had cleared from all tissues/fluids by day 7. *Beauveria bassiana* strain GHA is of low toxicity and is not pathogenic in the CD rat via the intratracheal route. The 14-day pulmonary LD_{50} is > 1.01×10^8 CFU/animal (males and females).

In an acute intraperitoneal injection study, no mortalities and no significant toxicity were observed in CD rats following intraperitoneal injection of *B. bassiana* strain GHA in 0.1% Tween 80 solution at 1.04×10^7 colony forming units per animal. Test substance had cleared from all treated animals by day 3. Based on these results, *B. bassiana* strain GHA is not infective and not pathogenic by intraperitoneal injection.

In an acute dermal toxicity study, there were no mortalities or signs of overt toxicity observed in New Zealand white rabbits following dermal exposure to 2 g of *B. bassiana* strain GHA per animal (1.6×10^{11} CFU/animal; 0.56 g/kg bw) for 24 hours. Signs of dermal irritation were observed in all rabbits within one hour after removal of wrappings and were still present as red spots on 5 rabbits at the end of the 14-day observation period. The 14-day dermal LD_{50} of *B. bassiana* strain GHA in rabbits was > 0.56 g/kg bw (males and females; mean measured dose). As per human health and safety testing requirements, dermal toxicity testing should be conducted with the end-use product. Also, the test dose was well below recommended dose level of 2 g/kg bw, and the study was terminated before all clinical signs had cleared. Therefore, the study was considered supplemental.

In a primary dermal irritation study, Mycotrol Botanigard ES (1.21×10^{10} conidia/g) was minimally irritating to the skin (maximum irritation score [MIS] at 1 h # 0.333) when New Zealand white rabbits were dermally exposed to 0.5 g of undiluted Mycotrol Botanigard ES for four hours and scored for irritation.

In a primary dermal irritation study, Mycotrol Botanigard 22 WP (4.52×10^{10} spores/g) was mildly irritating to the skin in New Zealand white rabbits dermally exposed to 0.5 g of a paste for 4 hours. The MIS (1 h) was 1.833. Very slight to well defined erythema (all animals) and very slight edema (three animals) was observed, but all animals fully recovered by the 72-hour scoring period. Therefore, the principal display panel of the label must include the signal words "CAUTION - SKIN IRRITANT", and the secondary display panel must include the precautionary statements "May irritate skin" and "Avoid contact with skin."

Although the dermal toxicity study did not entirely meet guideline criteria, an adequate assessment of the potential for dermal toxicity from Botanigard ES and Botanigard 22 WP was possible based on the following information:

- *B. bassiana* strain GHA is not conducive to growth at the temperature of the human body;
- *B. bassiana* strain GHA demonstrated low infectivity via acute oral, pulmonary and intraperitoneal toxicity routes of exposure;
- in open literature, clinical cases of deep tissue infection from *B. bassiana* occurred only in severely immunocompromised patients; and
- there have been no work-related injuries/incidents reported at Mycotech Corporation's manufacturing/formulating facility in the year 2000–2001.

Based on its classification as a mild dermal irritant, signal words and precautionary statements will be required on the Botanigard 22 WP label to warn users of the potential for dermal irritation. Standard personal protective equipment will also mitigate against risk of dermal toxicity from formulants by limiting dermal exposure.

For Botanigard ES, no signal words or precautionary statements are required given the dermal irritation testing results. Based on a review of the dermal toxicity data available for the formulants in Botanigard ES, dermal toxicity is not expected. However, certain formulants in Botanigard ES are proprietary mixtures which contain components for which toxicity categories are not assigned. Based on information provided on the Material Safety Data Sheets for these mixtures and given that the maximum level of any single component from these mixtures is <3.0%, it is not expected that dermal toxicity will result from exposure to these formulants. Nevertheless, to protect human health, the label for Botanigard ES will include the same precautionary statements with respect to dermal irritation and the same standard personal protective equipment as Botanigard 22 WP, which will minimize the potential for dermal exposure. With these mitigative measures in place on both end-use product labels, it is not expected that the use of Botanigard ES and Botanigard 22 WP will result in an unacceptable risk to human health with respect to dermal toxicity. Therefore, replacement dermal toxicity studies with the end-use products are not required.

In a skin sensitization study following the Buehler method, young adult male guinea pigs were dosed topically once weekly with 0.08 g of *B. bassiana* strain GHA (8.2×10^9 spores/application) for 3 weeks (induction phase). A negative control group was left untreated. No positive skin reactions were observed during the induction phase in the treated or in the positive control group hexylcinnamaldehyde. Two weeks following the third induction dose, the test substance group and the negative control received a 0.08 g challenge dose of *B. bassiana* strain GHA by topical application. No mortalities occurred in any group, and no positive skin reactions were observed in either the treated or negative control group. The study was classified as unacceptable because the dose used in the induction phase was below the level required to illicit mild irritation and the test substance was not moistened during dosing. Note that a replacement study is not required because the PMRA does not require a skin sensitization study for microbial pest control agents as all microorganisms contain substances that can elicit allergic reactions.

In an eye irritation study on New Zealand white albino rabbits, 0.1 g of undiluted *B. bassiana* Technical was instilled into the everted lower lid of one eye (1.23×10^{10} conidia/eye) for 24 hours and scored for irritation. Corneal opacity and iridal irritation, accompanied by corneal epithelial peeling, was observed in two animals. Moderate conjunctival irritation and positive irritation reactions were also observed in all six animals. All treated eyes were clear of positive reactions 72 hours after treatment and had returned to a normal appearance by Day seven. Based on the MIS (1 h) of 13.3, the MAS of 6.8 and the occurrence of corneal epithelial peeling, the technical grade active ingredient is mildly irritating to the eyes. The principal display panel of the label of the technical class product must include the statement "CAUTION - EYE IRRITANT." The secondary display panel must include the statements "May irritate eyes." and "Avoid contact with eyes."

In a primary eye irritation study, Mycotrol ES9601 (1.21×10^{10} conidia/g) was found to be mildly irritating to the eyes based on the MIS (1 h) of 17.5, the MAS of 6.1, the occurrence of corneal epithelial peeling in one animal and a prolonged pain response after treatment following administration of 0.1 mL of undiluted product into the everted lower lid of the right eye of albino rabbits for 24 hours. The principal display panel of the label must include the statement "CAUTION - EYE IRRITANT", and the secondary display panel must include the statements "May irritate eyes" and "Avoid contact with eyes."

In a primary eye irritation study, Mycotrol WP 9616b (4.52×10^{10} spores/g) was moderately irritating to the eye after 0.1 g of test substance was placed into the everted lower lid of one eye of New Zealand white rabbits for 24 hours. Corneal opacity in five animals (severe in one), and iridal irritation and moderate to severe conjunctival irritation in all animals was observed, but these symptoms had resolved by Day 10. One animal had a pannus formation that did not resolve until Day 14. The MIS (24 h) was 32.2, and the MAS was 18.1. The principal display panel of the label must include the statement "WARNING - EYE IRRITANT" and the secondary display panel include the statements "Causes eye irritation" and "DO NOT get in eyes."

Higher tier subchronic and chronic toxicity studies were not required because of the low acute toxicity of the MPCA and no indications of infectivity, toxicity or pathogenicity in the test animals treated in the Tier I acute oral and pulmonary toxicity/infectivity tests.

A bacterial reverse mutation assay performed with *B. bassiana* strain GHA conidia spores using bacterial mutant strains of *Salmonella typhimurium* and *Escherichia coli* did not show substantial increase in revertant colony numbers in a plate-incorporation assay (pre-experiment) at a series of doses ranging from 3 to 5000 µg/plate in the presence or absence of metabolic activation. There was a substantial increase in revertant colony numbers in a pre-incubation assay (main test) at a series of doses ranging from 1–2500 µg/plate in the presence or absence of metabolic activation. However, given that a plate test assay is inappropriate for testing microbial agents, the study was classified as unacceptable.

Within the available scientific literature, there are no reports that suggest *B. bassiana* has the potential to cause adverse effects on the endocrine system of animals. The submitted toxicity/infectivity studies in the rodent indicate that, following oral and pulmonary routes of exposure, the immune system is still intact and able to process and clear the MPCA. Based on the weight of evidence of the available data, no adverse effects to the endocrine or immune systems are anticipated for *B. bassiana* strain GHA.

Certain strains of *B. bassiana* are known to produce metabolites, such as beauvericin, bassianolide, bassiacridin and oosporein. Acceptable methods to quantify beauvericin, a cyclic depsipeptide, in the technical product have been submitted (refer to Section 2.4). Toxicological testing with a batch of the technical grade active ingredient showing maximum levels of beauvericin demonstrated no significant toxicity or pathogenicity via the acute oral, pulmonary and intraperitoneal routes of exposure. Environmental toxicity testing on aquatic invertebrates (*Daphnia magna*) and freshwater fish (*Pimephales promelas*) with the same lot also showed no

significant toxicological effects. Therefore, the applicant proposes to monitor all future production batches to ensure the metabolite beauvericin does not exceed these levels.

3.2 Occupational / Bystander Exposure and Risk Assessment

3.2.1 Occupational

When handled according to the label instructions, there is potential for dermal, eye and inhalation exposure for applicators, mixer/loaders, handlers and early-entry workers, with the primary source of exposure to workers being dermal. Given unbroken skin is a natural barrier to microbial invasion of the human body, dermal absorption could occur only if the skin were cut, if the microbe were a pathogen equipped with mechanisms for entry through or infection of the skin, or if metabolites were produced that could be dermally absorbed. *Beauveria bassiana* has not been identified as a dermal wound pathogen, and there is no indication that it could penetrate the intact skin of healthy individuals.

As Botanigard 22 WP is a mild dermal irritant, precautionary label statements identifying the product as a skin irritant are required. Compliance with the required personal protective equipment (PPE) listed on the end-use product label and with a 12-hour restricted-entry interval (REI) will mitigate the potential for dermal irritation and dermal toxicity by minimizing dermal exposure. Botanigard ES was found to be minimally irritating to the skin. However, to cover off any potential dermal irritation from formulants in Botanigard ES and protect human health, precautionary label statements and standard PPE will be required on the Botanigard ES label. Early-entry workers will also be restricted from entering areas where Botanigard ES and Botanigard 22 WP have been applied for a period of 12 hours unless wearing the indicated PPE. With these mitigative measures, the use of Botanigard ES or Botanigard 22 WP is not expected to result in an unacceptable risk to human health with respect to dermal toxicity.

As *Beauveria bassiana* Technical and Botanigard ES are mild eye irritants, precautionary label statements are required to ensure that users are aware of the potential hazard. However, as Botanigard 22 WP is a moderate eye irritant, in addition to precautionary label statements, applicators, mixer/loaders, handlers and early-entry workers will be required to wear eye goggles to minimize eye exposure to Botanigard 22 WP. These label restrictions will adequately protect populations that are likely to be primarily exposed to the product.

While submitted studies on *B. bassiana* strain GHA did not indicate a pulmonary risk, inhalation exposure can be minimized if applicators and early-entry workers wear National Institute for Occupational Safety and Health (NIOSH) approved respirators (with any N-95, P-95, R-95 or HE filter).

The PMRA assumes that all microorganisms contain substances that can elicit positive hypersensitivity reactions, regardless of the outcome of sensitization testing. Label statements (i.e. Potential Sensitizer) and risk mitigation measures such as PPE including gloves, long sleeved shirts, long pants, NIOSH approved respirators (with any N-95, P-95, R-95 or HE filter), shoes and socks are required to minimize exposure and protect handlers and early-entry workers.

3.2.2 Bystander

Bystander exposure to the MPCA is not expected to pose an undue risk on the basis of the low toxicity and pathogenicity profile for *B. bassiana* strain GHA and the assumption, precautionary label statements will be followed when using Botanigard ES and Botanigard 22 WP.

The label does not allow applications outside of commercial greenhouses, therefore, non-occupational dermal exposure and risk to adults, infants and children are low. As the use sites are agricultural, exposure of infants and children in school, residential and daycare facilities is likely to be minimal to non-existent. Consequently, the health risk to infants and children is expected to be negligible.

3.3 Dietary Exposure and Risk Assessment

3.3.1 Food

While the proposed use pattern may result in some dietary exposure with possible residues in or on agricultural commodities, negligible to no risk is expected for the general population, including infants and children, or animals because *B. bassiana* strain GHA demonstrated no pathogenicity, infectivity or oral toxicity at the maximum dose tested in the Tier I acute oral toxicity and infectivity study. Toxicological testing with formulated end-use products equivalent to Botanigard ES and Botanigard 22 WP also indicated low oral toxicity.

Although a secondary metabolite of *B. bassiana* strain GHA, beauvericin, has been identified in the technical product, there was no significant toxicity and no signs of pathogenicity observed when rats were administered a batch of the technical product containing maximum levels via oral, pulmonary or intraperitoneal routes, or when aquatic organisms were exposed in solution. The registrant proposes to monitor all future production batches to ensure that levels of this metabolite in the technical product do not exceed this level. Furthermore, calculations that accounted for the dilution rates and typical application rates, and the fact that no additional metabolite is likely to be produced after application, indicate that maximum potential levels of beauvericin on any treated food commodity would be negligable. Therefore, the risks from secondary metabolites to the general population, including infants and children, or animals are also negligible.

Higher tier subchronic and chronic dietary exposure studies were not required because of the low toxicity of the MPCA and no indications of infectivity, toxicity or pathogenicity in the test animals treated in the Tier I acute oral and pulmonary toxicity and infectivity studies. Therefore, there are no concerns for chronic risks posed by dietary exposure of the general population and sensitive subpopulations, such as infants and children.

3.3.2 Drinking Water

The likelihood that *B. bassiana* strain GHA could enter neighbouring aquatic environments as a result of greenhouse use is negligible. No risks are expected from exposure to this microorganism

via drinking water because exposure will be minimal. No harmful effects were observed in the animals that were exposed orally in Tier I acute oral toxicity and infectivity testing. The labels for Botanigard ES and Botanigard 22 WP instruct users not to contaminate irrigation or drinking water supplies or aquatic habitats by cleaning equipment or disposal of wastes. Users are also required not to allow effluent or runoff from greenhouses containing this product to enter lakes, streams, ponds or other waters. Furthermore, municipal treatment of drinking water will likely remove the transfer of residues to drinking water. Therefore, potential exposure to *B. bassiana* strain GHA in surface and drinking water is negligible.

3.3.3 Acute and Chronic Dietary Risks for Sensitive Subpopulations

Calculations of acute reference doses (ARFDs) and acceptable daily intakes (ADIs) are not usually possible for predicting acute and long-term effects of microbial agents in the general population or to potentially sensitive subpopulations, particularly infants and children. The single (maximum hazard) dose approach to testing MPCAs is sufficient for conducting a reasonable general assessment of risk if no significant adverse effects (i.e. no acute toxicity, infectivity or pathogenicity endpoints of concern) are noted in acute toxicity and infectivity tests. Based on all available information and hazard data, the PMRA concludes that *B. bassiana* strain GHA is of low toxicity, is not pathogenic or infective to mammals, and that infants and children are likely to be no more sensitive to the MPCA than the general population. Thus, there are no threshold effects of concern and, as a result, no need to require definitive (multiple dose) testing or apply uncertainty factors to account for intraspecies- and interspecies variability, safety factors or margins of exposure. Further factoring of consumption patterns among infants and children, special susceptibility in these subpopulations to the effects of the MPCA, including neurological effects from prenatal or postnatal exposures, and cumulative effects on infants and children of the MPCA and other registered microorganisms that have a common mechanism of toxicity, does not apply to this MPCA. As a result, the Agency has not used a margin of exposure (safety) approach to assess the risks of *B. bassiana* strain GHA to human health.

3.4 Maximum Residue Limits

No significant adverse effects were reported in Tier I acute toxicity/pathogenicity studies with *B. bassiana*. A secondary metabolite of *B. bassiana* strain GHA has been identified in the technical product; however, no significant toxicity was observed when rats were administered a batch of the technical product containing maximum levels of the metabolite via oral, pulmonary or intraperitoneal routes, or when aquatic organisms were exposed in solution. All future production batches will be monitored to ensure that levels of this metabolite does not exceed this maximum level. Furthermore, taking into account the dilution rates of the end-use product and typical application rates and given that it is not expected that *B. bassiana* strain GHA would be actively growing and producing secondary metabolites once applied to the crops, the maximum potential levels of beauvericin on any treated food commodity is expected to be negligble. Therefore the establishment of an MRL is not required for *B. bassiana* strain GHA under section 4(d) of the *Food and Drugs Act* (adulteration of food) as defined under Division 15, section B.15.002 of the Food and Drugs Regulations. The *Food and Drugs Act* prohibits the sale of adulterated food, which is food containing a pesticide residue that exceeds the established MRL.

Pesticide MRLs are established for *Food and Drugs Act* purposes through the evaluation of scientific data under the *Pest Control Products Act*. Each MRL value defines the maximum concentration in parts per million (ppm) of a pesticide allowed in/on certain foods. Food containing a pesticide residue that does not exceed the established MRL does not pose an unacceptable health risk.

3.5 Aggregate Exposure

Based on the toxicity and infectivity test data submitted and other relevant information in the PMRA's files, there is reasonable certainty no harm will result from aggregate exposure of residues of *B. bassiana* strain GHA to the general Canadian population, including infants and children, when the microbial pest control product is used as labelled. This includes all anticipated dietary (food and drinking water) exposures and all other non-occupational exposures (dermal and inhalation) for which there is reliable information. Given the product is to be used in greenhouses and is not allowed for use on turf, residential or recreational areas, dermal and inhalation exposure to the general public will be very low. Furthermore, few adverse effects from exposure to natural populations of *B. bassiana* in the environment have been reported. Even if there is an increase in exposure to this microorganism from the use of Botanigard ES and Botanigard 22 WP, there should not be any increase in potential human health risk.

3.6 Cumulative Effects

The PMRA has considered available information on the cumulative effects of residues and other substances that have a common mechanism of toxicity. These considerations included the cumulative effects on infants and children. Besides naturally occurring strains of *B. bassiana* in the environment and one other strain of *B. bassiana* used as a fungicide in chicken houses (Balance ES – Registration Number 28890), the Agency is not aware of any other microorganisms or other substances that share a common mechanism of toxicity with this active ingredient. No cumulative effects are anticipated if the residues of *B. bassiana* strain GHA interact with related strains of this microbial species.

4.0 Impact on the Environment

4.1 Fate and Behaviour in the Environment

Beauveria bassiana is typically recovered from forest and cultivated soils and is a prominent species in soils from temperate and near-northern habitats in Canada. *B. bassiana* is considered to be a ubiquitous fungus that can also be isolated from running water, rodents and their nests, feathers and droppings of free-living birds. *B. bassiana* strain GHA originates from a natural specimen isolated from the Southern corn root worm (*Diabrotica undecimpunctata*) on green beans in Oregon United States.

The proposed use of Botanigard ES and Botanigard 22 WP is limited to greenhouses, where the product will be targeted to the foliar part of the crop while avoiding run-off from the plant onto the growing medium. Although the greenhouse uses preclude direct exposure to outdoor

environments, outdoor soils may be exposed to *B. bassiana* strain GHA through human activity, such as composting of plant waste and water management practices. Afterwards, the dispersal of *B. bassiana* strain GHA should be limited to run-off and natural vectors (e.g., insects). Based on these considerations, the amount of *B. bassiana* strain GHA transferring to outdoor environments as a result of the greenhouse use of Botanigard ES and Botanigard 22 WP is expected to be low. However, in the event that *B. bassiana* strain GHA should reach outdoor soil environments, the organism is expected to behave as it would in nature. As a ubiquitous soil microorganism, it is likely that *B. bassiana* would settle in the soil where it is commonly found, rather than percolate through soil. Therefore, mobility through the soil is expected to be minimal.

Beauveria bassiana is not an aquatic fungus and there are no reports in the published scientific literature of it ever having been isolated from aquatic environments. As the surfaces of fungal aerial conidia are hydrophobic, and the Botanigard formulations contain only conidia of *B. bassiana* strain GHA, any conidia that reach aquatic habitats are likely to cluster together at the surface where they would be quickly inactivated by sunlight.

Given the ubiquitous nature of *B. bassiana* as a soil microorganism, it is likely that *B. bassiana* strain GHA could survive in the soil under suitable environmental conditions (i.e. moisture, pH); however, over time, the population of *B. bassiana* strain GHA should return to naturally occurring levels. Persistence of *B. bassiana* strain GHA under field conditions is expected to be limited due mainly to a rapid decrease in conidial survival when exposed to sunlight. Like most fungal species, temperature, humidity and solar radiation are the most important environmental factors in its propagation and survival in the environment. The optimum temperature for *B. bassiana* is 23-28°C, with minimum and maximum ranging from 5–10°C and 30–38°C, respectively, depending on the isolate. Spore germination of *B. bassiana* on the insect cuticle and sporulation after outgrowth of the dead insect requires high moisture, generally in the range of 92–100%.

Physical stability studies suggest survival of conidia is also expected to be limited in aquatic environments. However, no raw data from the studies were submitted to allow for independent review.

4.2 Effects on Non-Target Species

4.2.1 Effects on Terrestrial Organisms

The ecotoxicology package submitted to address the risks of *B. bassiana* strain GHA to terrestrial organisms included testing on avian species, earthworms, various terrestrial arthropods, and a field study on various insect species. Effects on non-target plants were addressed via efficacy studies. A waiver request for avian oral pulmonary toxicity and wild mammal testing was not provided; it was considered acceptable given that information was available to adequately assess the risks to these non-target species. Summaries of the studies are discussed below. Refer to Appendix I, Table 2, for additional details.

The acute oral toxicity of *B. bassiana* strain GHA to avian species was assessed in a field study with native American kestrels (*Falco sparverius*) dosed with a single oral dose of Mycocide B GH Oil Flowable containing the microbial pest control agent *B. bassiana* strain GHA, by gelatin capsule at 5 µL/g body weight (bw; test 1: nominal: 2.5×10^7 conidia/g bw; N = 2) or by tuberculin syringe at 1 µL/g bw (test 2: nominal: 5×10^6 conidia/g bw, N = 13). The exact formulation of Mycocide B GH oil flowable was not stated. There were no mortalities at either dose level, no differences in growth rates compared to control groups and no visible gross pathology upon necropsy (test 2 only). Also, a published acute oral toxicity study with the Japanese quail (*Cortunix coturnix Japonica*; 2/sex) demonstrated that *B. bassiana* strain ATCC 26848 was not toxic or infective at 2.95×10^{10} spores/bird.

Avian pulmonary and wild mammal toxicity/infectivity testing was waived based on the minimal anticipated exposure from the intended greenhouse use, which will not significantly increase the level of *B. bassiana* strain GHA in the environment, and the fact that human health data performed on laboratory animals showed no detrimental effects to mammalian species. Furthermore, given that *B. bassiana* strain GHA is unable to grow at temperatures above 35°C, the MPCA will not proliferate at avian (40°C) or mammalian (37°C) body temperatures. In addition, there were no reports of adverse effects in wild birds or mammals from naturally occurring populations of *B. bassiana*. Therefore, no additional testing is required.

In a 14-day contact toxicity/pathogenicity study, earthworms (*Eisenia fetida*) were exposed to *B. bassiana* strain GHA in artificial soil substrate at one of five nominal doses ranging from 1.1×10^{10} to 8.6×10^{10} conidia/kg soil. The 14-day LC_{50} was > 1000 mg/kg, the highest concentration tested. The no observed effect concentration (body weight) was 1000 mg/kg.

The potential hazards to non-target terrestrial plants were addressed in the numerous efficacy studies conducted to support the proposed uses of Botanigard ES and Botanigard 22 WP (see Section 5.3 Impact on Succeeding Crops, Adjacent Crops and on Treated Plants). Some phytotoxicity was observed on certain plants during efficacy testing; therefore, advisory statements notifying users to spot test plant surfaces before applying product for the first time are required on the label under the directions for use section. Also included on the label will be instructions for users to avoid over-application to minimize the risk of build up of visible residues on plant surfaces and to avoid potential phytotoxic effects on crop plants. In a literature search of relevant databases, there were no reports of phytotoxicity due to *B. bassiana*, and no *Beauveria* species were identified as phytopathogens. Also, the greenhouse use of Botanigard ES and Botanigard 22 WP minimizes exposure to many non-target plants. Based on a weight of evidence and anticipated precautionary labelling, no additional terrestrial plant testing is required to support the proposed use of Botanigard ES and Botanigard 22 WP in greenhouse food and greenhouse non-food crops.

The potential hazards to non-target arthropods were addressed via studies testing the effects of *B. bassiana* strain GHA on various orders of beneficial or environmentally important insects, including flea beetle (*Apthona flava*), Warehouse Pirate bug (*Xylocoris flavipes*), parasitic wasp (*Eretmocerus* species) and mealworm beetle (*Tenebrio molitor*). A honeybee (*Apis mellifera*) hive study and a field study on non-target insects were also submitted. The non-target insect

studies did not meet guideline criteria in that exposure to the MPCA was not carried out at 90% relative humidity, viability of the test substance was not confirmed and infectivity was not unequivocally determined. Despite these limitations, the studies offer insight into the potential for adverse effects on non-target insects.

In a 10-day toxicity and pathogenicity/infectivity study, adult flea beetles (*Apthona flava*) were exposed to *B. bassiana* strain GHA in 0.05% Tween 80 by overhead spraying at 1 of 4 rates in a series ranging from 6.0 × 104 to 2.2 × 106 conidia/cm^2. Mortality of beetles in the treatment groups followed a dose-response trend over the 10-day period with a maximum mortality of 91% on day 10 in the highest test group. All cadavers from all dose levels showed the presence of *B. bassiana* strain GHA after incubation under high humidity. The acute (single exposure) contact exposure LD_{50} was 5.2×10^5 conidia/cm^2.

In a 10-day environmental exposure toxicity and pathogenicity/infectivity study, Warehouse pirate bug nymphs (*Xylocoris flavipes*) were exposed to *B. bassiana* strain GHA in 0.05% Tween 80 on filter paper discs in plastic vials to 1 of 3 concentrations in a series ranging from 2.6×10^5 to 2.7×10^7 conidia/cm^2. Given that Tween 80 is a detergent that may have insecticidal properties, a carrier control group should have been included. Significantly higher mortality was observed in insects treated with viable MPCA at levels $\geq 2.6 \times 10^6$ conidia/cm^2 compared to the attenuated control group, which can be unequivocally attributed to exposure to the MPCA alone. Fungal infection developed on all cadavers from all viable dose groups following incubation under high humidity.

In a study on the effects of *B. bassiana* strain GHA on insect parasitism, whitefly larvae (*Bemisia tabaci* on sweet potato leaves) previously exposed to the parasitic wasp (*Eretmocerus* n. sp.) for 1, 2, 3, 9 or 13 days, were treated with a single application of Mycotrol WP, containing *B. bassiana* strain GHA suspended in 0.01% Tween 80, at 1000 conidia/mL^2 (nominal) and incubated for 24 hours. No significant effect on parasitism was observed in larvae originally parasitized for two or more days. There was no effect on the fecundity of mated female parasitoids. However, treatment with Mycotrol WP significantly reduced the longevity of surviving adult parasitoids even when exposure occurred after 13 days of parasitization. Also, adult parasitoids (outside of a host) showed a significantly shorter longevity following direct spraying.

In a contact toxicity/pathogenicity study, mortality of mealworm beetles (*Tenebrio molitor*) exposed by simulated aerial application to *B. bassiana* strain GHA in Mycocide OF oil carrier at 2×10^4 conidia/spray or 2.4×10^8 conidia/spray did not considerably increase over a 10-day observation period compared to untreated control beetles or beetles treated with carrier oil alone. At the low rate, mortality in the carrier oil control group was the same as the formulated product, suggesting that the carrier oil may have insecticidal properties.

A field study was conducted to assess the risks to various non-target insects. Following application of Mycotrol ES at 1.75×10^{13} conidia/ha (rangeland), or 3.5×10^{13} conidia/ha (alfalfa), insects were collected from the fields and processed for *B. bassiana* strain GHA. Processing involved liberal estimates of internal levels of the MPCA by plating surface-sterilized

homogenates on semi-selective media or conservative estimates by incubating freeze-killed arthropods on moistened filter paper to monitor hyphal growth (i.e. colonization by the MPCA). Persistence of *B. bassiana* strain GHA conidia in the fields was monitored by plate counts from rangeland grasses and alfalfa leaf samples collected throughout the study.

At the rangeland site, *B. bassiana* strain GHA levels in insects and on rangeland grasses declined over time, suggesting that any impact from the MPCA on non-target rangeland insects would be minimal and short-lived. Also, grasshoppers collected from *B. bassiana*-treated fields were monitored for development of mycosis in cages under field or under greenhouses conditions and showed a faster development of mycosis, and a much higher incidence of final mycosis, under greenhouse conditions. The apparent resistance of field grasshoppers to infection can be correlated to the grasshopper's basking behaviour, which results in thermally 'curing' themselves of *B. bassiana* strain GHA infection.

At the alfalfa site, certain non-target insects (e.g., Harvestmen spiders, Ladybird beetles, Alfalfa weevil, Lygus bugs, Alfalfa plant bugs) did develop fungal infections following exposure to *B. bassiana* strain GHA under field conditions. In particular, twenty percent of Alfalfa leafcutting bees collected from hives within the alfalfa test plots 10 days post-application had a high prevalence of *B. bassiana* strain GHA accompanied by overt signs of infection when incubated on moistened filter paper. There were no observed effects on survival of bee larvae, pre-pupae, or adult emergence following overwintering of bees. These findings indicate that certain non-target insect species could be at risk of developing infection from the MPCA particularly if conditions promote fungal infection (i.e. high humidity).

In a contact toxicity and infectivity/pathogenicity study, worker honeybees (*Apis mellifera*) were treated with Mycotrol WP, containing the biological control agent *B. bassiana* strain GHA suspended in 0.01% Silwet L77, at a nominal dose of 2.64×10^8 spores/mL per spray (measured: 2.27×10^5 spores/bee) by simulated field application. A carrier control group was not included but was considered acceptable given the generally perceived safety of Silwet L-77 to honeybees. Although wetting agents have the tendency to result in an unintentional reduced exposure, the measured dose per bee indicate that a considerable amount was successfully delivered. Visual observation of graphical data showed no drastic difference in mortality between worker bee groups over the 30-day observation period but definitive quantification was not possible as data were only presented graphically. The overall prevalence of infection in worker bees was determined as the proportion of dead bees testing positive for the MPCA out of the total number of bees exposed directly, or indirectly, and was calculated to be 2.1% with no observed latent or prepatent infections in worker bees sacrificed at test termination. There were no signs of fungal infection by *B. bassiana* strain GHA in the larval brood and no changes in normal homeostatic processes within colonies. Statistical analyses of the larval brood survival data were meaningless, and an LC_{50} was not calculated. The overall low prevalence of infection observed in working bee hives and broods can be attributed to normal hive temperatures (32–36°C) and normal thoracic temperature during active states (30°C) which are sufficiently high to reduce or even inhibit growth of *B. bassiana* strain GHA, thus minimizing its impact on bees even in the event of direct exposure.

Conflicting information was available in studies on the effects of Botanigard ES and Botanigard 22 WP to bumblebees (*Bombus impatiens*) and honeybees (*Apis mellifera*). A high mortality (>50%) was observed in bees in colonies following treatment with Botanigard ES and Botanigard 22 WP by immersion or air-brush spraying, and *B. bassiana* strain GHA showed among the highest level of mortality to honeybees compared to other strains tested under laboratory conditions. However, preliminary studies with bumblebees as biological control vectors of *B. bassiana* strain GHA at concentrations up to 2.0×10^{11} conidia/g showed no adverse effects on pollination activity or the number of bees in hives in greenhouses. The inoculum was prepared by mixing corn flour with Botanigard 22 WP, which was added to an inoculum dispenser and attached to a bee hive for delivery to the bees (no further details provided). Together, the reports suggest that honeybees could be harmed if directly exposed under the worst-case scenario conditions, but that bumblebees specifically may be unaffected under operational conditions of use.

As the proposed uses of Botanigard ES and Botanigard 22 WP are limited to greenhouses, there is no direct exposure to outdoor environments, and outside environments are only expected to be exposed to *B. bassiana* strain GHA through operational activity (e.g., removal and composting of spent crop growing media). Once outside, the dispersal of *B. bassiana* strain GHA should be limited to mostly runoff and vectors. Based on these considerations, the amount of *B. bassiana* strain GHA transferring to outdoor environments is expected to be low. Consequently, a significant increase in natural populations of *B. bassiana* in outdoor terrestrial environments is not expected, and hazards to non-target terrestrial organisms are expected to be minimal to non-existent. However, the greenhouse use of Botanigard ES and Botanigard 22 WP warrants particular attention with respect to the use of beneficial insects in integrated pest management programs, particularly since the MPCA has demonstrated commercial relevance against various insect orders or families, including Homoptera (e.g., whitefly, *Bemisia* spp., *Trialeurodes vaporarium*; aphid, *Myzuz persicae*, *Aphis gossypii*; leafhopper, *Erythoneura elegantula*), Thysanoptera (e.g., thrips, *Frankliniella occidentalis*, *Thrips palmi*), Acrididae (e.g., migratory grasshopper *Melanoplus sanguinipes*, North American grasshopper, African grasshopper, and locusts) and Lepidoptera (e.g., diamondback moth, *Plutella xylostella*, imported cabbage worm, *Pieris rapue*; cabbage looper, *Trichoplusia ni*). Non-target insect laboratory tests such as those submitted in the ecotoxicology package for *B. bassiana* strain GHA reflect conservative measures of the potential for effects. Successful infection by *B. bassiana* and spore germination requires suitable environmental conditions (e.g., high moisture ca. 92–100%) which may or may not be reached in greenhouse settings. Other factors, such as behaviour of insects (e.g., insect emergence, insect movement), seasonality, and habitat of insect species would further limit exposure and thus mitigate risks to non-target insects. Nevertheless, based on the results from non-target insect testing and the known entomopathogenic nature of *B. bassiana* strain GHA, it is reasonable to conclude that certain insect species may be adversely affected in the event of direct exposure to Botanigard ES and Botanigard 22 WP under greenhouse conditions particularly when maximum application rates and repeat intervals are followed. Therefore, to reduce the risks to non-target insects the labels for Botanigard ES and Botanigard 22 WP will instruct users to minimize overspray in the greenhouse. Furthermore, precautionary labelling will state that Botanigard ES and Botanigard 22 WP may be harmful to beneficial insects, and that direct contact with beneficial insects should be avoided. To ensure that honeybees are not adversely

affected, precautionary labelling will also specifically instruct users to avoid spraying when bees are actively foraging.

Data were not required to address the risks of *B. bassiana* strain GHA to soil microorganisms, as *B. bassiana* is a natural component of soils, and it is unlikely that such a ubiquitous soil microorganism would destabilize non-target soil or plant-associated microbial populations.

4.2.2 Effects on Aquatic Organisms

Studies submitted to address the hazards of *B. bassiana* strain GHA to aquatic non-target organisms included testing on freshwater fish, daphnids and algae.

In a 31-day chronic early life-stage toxicity/pathogenicity and infectivity study, fertilized Fathead minnow (*Pimephales promelas*) eggs were exposed to *B. bassiana* strain GHA in solution at a single maximum hazard concentration of 7.5×10^8 conidia/L (mean measured) under static renewal conditions. After hatching, surviving fry were transferred to respective retention chambers for observation. There were no effects on day-to-hatch, or percent hatch of embryos. Survival of larvae was highest in the treatment group. *Beauveria bassiana* strain GHA was not infective or pathogenic to fathead minnow by aqueous exposure and the 31-day LC_{50} was $> 7.5 \times 10^8$ conidia/L (mean measured). Growth of fathead minnows (total length and total wet weight) from the treatment group was significantly reduced after test termination, but the biological significance of this finding was uncertain as feeding rates were not adjusted for the increased survival in the treatment group.

In a 21-day toxicity/pathogenicity study, groups of daphnids (*Daphnia magna*) were exposed to *B. bassiana* strain GHA in the test water under static renewal conditions at one of five mean measured doses ranging from 6.4×10^7–9.3×10^8 spores/L. The 21-day EC_{50} based on survival was $> 9.3 \times 10^8$ spores/L (mean measured), the highest concentration tested. The 21-day NOEC and LOEC, based on length, were 4.7×10^8 spores/L (mean measured) and 9.3×10^8 spores/L (mean measured), respectively.

In a 96-hour acute toxicity test, freshwater algae (*Selenastrum capriconutum*, 1×10^4 cells/mL) were exposed to *B. bassiana* strain GHA at one of five test concentrations in a geometric series ranging from 19–300 mg/mL (mean measured: 1.48×10^6–2.57×10^7 spores/mL; 80–113% of nominal) under static conditions. Algal growth measurements (as cell densities) were used to calculate area under the growth curve values and growth rates, which, in turn, were used to calculate percent inhibition values relative to the control over 96 hours. The 96-hour EC_{50} (cell densities), EbC_{50} (biomass), and ErC_{50} (growth rate) were determined to be 118 mg/L, 115 mg/L, and 299 mg/L, respectively. The NOEC, based on cell density, area under the growth curve and growth rate, was 75 mg/L. All calculations were based on nominal concentrations.

Two published studies reported adverse effects from *B. bassiana* to embryos of inland silverside fish. Nevertheless, hazards to non-target aquatic organisms from the greenhouse use of Botanigard ES and Botanigard 22 WP are expected to be minimal to non-existent based on the following considerations: the anticipated outdoor exposure from the greenhouse use of

Botanigard ES and Botanigard 22 WP is expected to be minimal; *B. bassiana* strain GHA is likely to settle in the soil where it is commonly found, rather than leach through the soil to aquatic environments; and no *Beauveria* species or its phylogenetically close relatives have any relevance in aquatic animal disease. Standard label statements for greenhouse pest control products instruct users not to allow effluent from greenhouses to enter lakes, streams, ponds or other water bodies and are considered adequate to protect aquatic environments. Based on these considerations, the greenhouse use of Botanigard ES and Botanigard 22 WP is expected to pose a minimal impact on non-target aquatic organisms.

5.0 Value

5.1 Effectiveness Against Pests

5.1.1 Acceptable Efficacy Claims

5.1.1.1 Control of Thrips

The proposed application rate for thrips is 5mL/L (0.5%) for Botanigard ES and 1.25 to 2.5 g/L (0.125% to 0.25%) for Botanigard 22 WP. Rates tested for control of thrips for Botanigard ES included 0.125%, 0.25% and 0.5%, while rates tested for Botanigard 22 WP ranged from 0.625 g/L to 1.25 g/L (0.0625% to 0.125%). A total of four trials were reviewed to support a control claim of thrips on greenhouse vegetables and ornamentals; two of the trials tested Botanigard 22 WP and three tested Botanigard ES. Two trials were on ornamentals and two were on greenhouse vegetables (cucumber and tomato). In one of the ornamental trials (ficus, rose, Saintpaulia, chrysanthemum), no significant difference was demonstrated between the control and the treatments. The remaining ornamental trial on chrysanthemum demonstrated that Botanigard 22 WP could give a high level of control, up to 93% at application rates of 0.625 to 1.25 g/L.

The small-scale greenhouse trial on cucumber demonstrated that, given ideal (high) greenhouse humidity levels, the rate of western flower thrips (adults and immature) infected when Botanigard ES was applied at 5 mL/L could be as high as 90%. The commercial greenhouse trial on cucumber and tomato also demonstrated high levels of infection when Botanigard ES was applied at 5 mL/L for control of western flower thrips (from 40% to 89%). In general, the data indicated that lower humidity levels (e.g., 75–80%) resulted in much lower infection rates than high relative humidity levels (89–97%).

5.1.1.2 Control of Whitefly

The proposed application rate for whitefly is 1.25 to 2.5 mL/L for Botanigard ES and 0.625 to 1.25 g/L for Botanigard 22 WP. Rates tested were 1.25 and 2.5 mL/L for Botanigard ES and 0.625 and 1.25 g/L for Botanigard 22 WP. Whitefly species tested included greenhouse whitefly, silverleaf whitefly, and sweet potato whitefly. Botanigard 22 WP was tested in seven trials on greenhouse ornamentals and four on greenhouse tomatoes. Botanigard ES was tested in five trials on greenhouse ornamentals and in three on greenhouse tomatoes. While both formulations of

Botanigard generally reduced the number of whiteflies, with levels of control ranging up to 93%, control was variable, most likely due to variations in temperature and humidity. One study on control of whitefly on ornamentals demonstrated that Botanigard ES performed significantly better than Botanigard 22 WP (83–95% mortality vs. 63–76% mortality, respectively). Some evidence was seen for a rate effect where the low rate and high rate were tested side-by-side.

5.1.1.3 Control of Aphids

The proposed application rate for aphids is 1.25 to 2.5 mL/L for Botanigard ES and 0.625 to 1.25 g/L for Botanigard 22 WP. One study was provided which tested Botanigard ES at 5.0 mL/L for control of melon aphids and green peach aphids on cucumber. Infection rates after treatment were up to 96%. No data were provided that tested the efficacy of these products at the proposed application rates. However, given the mode of action of Botanigard ES and 22 WP, the application methods (frequent repeated applications until control is achieved), the data that demonstrated that aphids are susceptible to *Beauvaria bassiana*, and the fact that the proposed rates were demonstrated to be effective against other greenhouse pests, it is reasonable to assume that control of aphids would be likely at the proposed application rates.

5.1.1.4 Efficacy Conclusions

The data provided for control of thrips and whiteflies on greenhouse ornamentals for Botanigard ES and Botanigard 22 WP are sufficient to support the proposed use against these pests. While efficacy was variable and did not always provide good control, this was not unexpected given the mode of action of these products (biological control agent which causes a disease). Differences in humidity and temperature can result in large differences in efficacy, including complete control failure. While no clear, consistent differences wereseen between low and high application rates, it is reasonable to include an application rate range to allow the user some flexibility in application given the wide variety of crops to which these products will be applied (greenhouse vegetables and ornamentals).

For greenhouse vegetables, data were only provided for tomato and cucumber. However, a wide variety of ornamentals were tested. Given the mode of action, it is likely that Botanigard ES and 22 WP would be effective independent of the greenhouse crop which is treated. As the proposed application rates are given as a concentration, the most important issue is proper coverage.

The acceptable efficacy claims for Botanigard 22 WP are control of whiteflies and aphids at an application rate of 0.625 to 1.25 g/L spray (0.14 to 0.28 g a.i.) and control of thrips at an application rate of 1.25 to 2.5 g/L spray (0.275 to 0.55 g a.i.).

The acceptable efficacy claims for Botanigard ES are control of whiteflies and aphids at an application rate of 1.25 to 2.5 mL/L spray (0.14 to 0.28 mL a.i.) and control of thrips at an application rate of 5 mL/L spray (0.56 mL a.i.).

5.2 Phytotoxicity to Target Plants

5.2.1 Botanigard 22 WP

Botanigard 22 WP, especially at the higher rates, often resulted in commercially unacceptable visible residues. A warning is required on the label that states "CAUTION: Use of Botanigard 22 WP, especially at higher application rates, may result in commercially unacceptable visible residues. To minimize the risk of unacceptable visible residues on crop, test product by treating a small portion of the crop to determine if visible residues are acceptable."

5.2.2 Botanigard ES

Botanigard ES resulted in phytotoxic damage to some varieties of tomato plants. The phytotoxic effects of Botanigard ES on greenhouse vegetables other than tomatoes and cucumbers is unknown. No phytotoxic effects were observed on greenhouse ornamentals treated with Botanigard ES. A warning is required on the label which states "CAUTION: Botanigard ES has been observed to cause foliage damage in some varieties of plants (necrotic spots on leaves in some varieties of tomatoes). To minimize the risk of foliar damage to crop, before using on a new variety of plant, test product by treating a small portion of the crop and observe for injury or damage."

5.3 Impact on Succeeding Crops, Adjacent Crops and on Treated Plants

Not applicable.

5.4 Economics

Not assessed.

5.5 Sustainability

5.5.1 Survey of Alternatives

Table 5.5.1 List of Active Ingredients Currently Registered on Greenhouse Vegetables and/or Ornamentals for Aphids, Whitefly and Thrips

Pest	Insecticide Active Ingredients
Aphids	nicotine diazinon acephate endosulfan malathion insecticidal soap bendiocarb

Pest	Insecticide Active Ingredients
	chlorpyrifos dichlorvos (S)-konoprene imidacloprid acetamiprid pymetrozine naled
Whitefly	methomyl acephate endosulfan malathion insecticidal soap permethrin deltamethrin bendiocarb chlorpyrifos dichlorvos (S)-konoprene imidacloprid acetamiprid pymetrozine naled pyridaben pyriproxyfen spiromesifen
Thrips	nicotine acephate malthion bendiocarb chlorpyrifos deltamethrin dichlorvos spinosad

5.5.2 Compatibility with Current Management Practices Including Integrated Pest Management

While use of the entomopathogenic fungi *Beauvaria bassiana* strain GHA is consistent with standard preventative integrated pest management practices for greenhouse vegetables and ornamentals, as this product is a fungal biological control agent, fungicides and other pesticides may kill the spores.

5.5.3 Information on the Occurrence or Possible Occurrence of the Development of Resistance

Due to the mode of action of Botanigard ES and Botanigard 22 WP (generalist entomopathogenic fungi), resistance to these products is not expected to develop.

6.0 Pest Control Product Policy Considerations

6.1 *Toxic Substances Management Policy* Considerations

The management of toxic substances is guided by the federal government's *Toxic Substances Management Policy*, which puts forward a preventive and precautionary approach to deal with substances that enter the environment and could harm the environment or human health. The policy provides decision makers with direction and sets out a science-based management framework to ensure that federal programs are consistent with its objectives. One of the key management objectives is virtual elimination from the environment of toxic substances that result predominantly from human activity and that are persistent and bioaccumulative. These substances are referred to in the policy as Track 1 substances.

While reviewing *B. bassiana* strain GHA, the PMRA took into account the federal Toxic Substances Management Policy and followed its Regulatory Directive DIR99-03, The Pest Management Regulatory Agency's Strategy for Implementing the Toxic Substances Management Policy. Substances associated with its use were also considered, including microcontaminants in the technical product, *Beauveria bassiana* Technical and formulants in the manufacturing-use products Botanigard ES and Botanigard 22 WP. The PMRA has reached the following conclusions:

- *Beauveria bassiana* strain GHA does not meet the Track 1 criteria because the active ingredient is a biological organism and hence is not subject to the criteria used to define persistence, bioaccumulation and toxicity properties of chemical control products. There are also no formulants, contaminants or impurities present in the end-use product that would meet the TSMP Track 1 criteria.

- *Beauveria bassiana* strain GHA does not contain any contaminants of health or environmental concern identified in *Canada Gazette* Part II, Volume 139, Number 24, pages 2641–2643: List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern.

Therefore, the use of *Beauveria bassiana* Technical is not expected to result in the entry of Track 1 substances into the environment.

6.2 Formulants or Contaminants of Environmental Concern

Two of the formulants in Botanigard ES and one of the formulants in Botanigard 22 WP contain petroleum distillates that are classified as List 2 formulants. While both end-use products were

classified as being of low acute toxicity by the oral route, users should be aware that these products contain petroleum distillates that pose a potential aspiration hazard. As per the Agency's First Aid Labelling Statements (DIR2007-01, First Aid Labelling Statements) all products containing any level of petroleum distillates must include additional precautionary label statements and first aid measures to identify the presence of petroleum distillates in the product. Furthermore, early-entry workers will also be restricted from entering areas where Botanigard ES and Botanigard 22 WP have been applied for a period of 12 hours unless wearing the indicated PPE. Botanigard ES specifically contains heavy aromatic petroleum distillates that are toxic to aquatic organisms; therefore, a precautionary statement to protect aquatic organisms will also be required on the end-use product label.

Although it does not appear on the List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern (*Canada Gazette*, Part II, Volume 139, Number 24, pages 2641–2643), a component of one of the formulants in Botanigard ES is considered to be toxic as defined in section 64 of the *Canadian Environmental Protection Act*, 1999. The PMRA has conducted a risk assessment of this formulant component and has found that the associated risk is acceptable for the proposed use.

A formulant in the end-use product Botanigard 22 WP contains sulfites that are identified in *Canada Gazette* Part II, Volume 139, Number 24, pages 2641–2643: List of Pest Control Product Formulants of Health or Environmental Concern as an allergen known to cause anaphylactic-type reactions. Therefore, the label for end-use product Botanigard 22 WP will include the precautionary statement "Warning: this product contains the allergen sulfites" on the principal display panel.

One of the formulants in Botanigard 22 WP contains a substance that has been included in the Government of Canada's Challenge to Industry arising out of the categorization of the Domestic Substances List. For more information on the Challenge, please consult the relevant documents on the PMRA's website at: www.pmra-arla.gc.ca/english/pdf/appregis/Memo-Post-categorization-e.pdf and www.pmra-arla.gc.ca/english/pdf/appregis/Pesticide-substances-in-Challenge-ef.pdf.

7.0 Summary

7.1 Methods for Analysis of the Microorganism as Manufactured

The product characterization data for *Beauveria bassiana* Technical, Botanigard ES and Botanigard 22 WP are adequate to assess their safety to human health and the environment. The technical material was fully characterized and the specifications were supported by the analysis of a sufficient number of batches.

Acceptable methods to monitor for the secondary metabolite beauvericin in the technical product have been submitted.

Storage stability data were sufficient to support an expiration date of nine months for Botanigard ES and Botanigard 22 WP when the products are stored at room temperature (25°C).

7.2 Human Health and Safety

The acute toxicity and infectivity studies submitted in support of *B. bassiana* strain GHA were determined to be sufficiently complete to permit a decision on registration. *B. bassiana* strain GHA was of low toxicity and was not infective in the rat when administered via the oral, pulmonary, and intraperitoneal routes. As *Beauveria bassiana* Technical is a mild ocular irritant, the signal words "CAUTION– EYE IRRITANT" and the precautionary statements "May cause eye irritation. Avoid contact with eyes." are required on the principal and secondary display panel, respectively, of the technical label.

Based on the lack of growth of the MPCA at the normal human body temperature; lack of infectivity of the MPCA by the oral, pulmonary and intraperitoneal route; lack of reports of dermal toxicity from *B. bassiana* strain GHA in healthy individuals in published literature; a history of safe use of Botanigard ES and Botanigard 22 WP in the United States since 2000 and precautionary label statements that will warn users to avoid dermal exposure, it is not expected that use of *Beauveria bassiana* Technical, Botanigard ES or Botanigard 22 WP will result in an unacceptable risk to human health with respect to dermal toxicity.

Botanigard ES and Botanigard 22 WP are both of low toxicity by the oral route. Botanigard ES was a minimal skin irritant and a mild ocular irritant. Consequently, the signal words "CAUTION– EYE IRRITANT" and the precautionary statements "May cause eye irritation. Avoid contact with eyes." are required on the principal and secondary display panel, respectively, of the Botanigard ES label. Additionally, in order to be protective of human health and to cover off any potential dermal irritation from formulants, precautionary label statements on the secondary display panel ("May cause skin irritation. Avoid contact with skin.") and standard PPE will be required on the Botanigard ES label to minimize dermal exposure.

Botanigard 22 WP is a mild skin irritant and a moderate ocular irritant. Consequently, the signal words "CAUTION–SKIN IRRITANT" and "WARNING–EYE IRRITANT" are required on the principal display panel of Botanigard 22 WP, as well as the statements "May cause skin irritation. Avoid contact with skin." and "Causes eye irritation. DO NOT get in eyes." on the secondary display panel of the product label.

Two formulants in Botanigard ES and one formulant in Botanigard 22 WP contain petroleum distillates that are List two formulants that pose a potential aspiration hazard; therefore, additional precautionary statements and first aid measures will be required on both end-use product labels. As Botanigard ES specifically contains heavy aromatic petroleum distillates that are toxic to aquatic organisms, a precautionary label statement to protect aquatic organisms will also be required.

A formulant in the end-use product Botanigard 22 WP contains sulfites that are identified as allergens known to cause anaphylactic-type reactions; therefore, the end-use product label for Botanigard 22 WP must include the precautionary label statements notifying users of the allergen. In addition, one of the formulants in Botanigard 22 WP contains a substance that has been included in the Government of Canada's Challenge to Industry arising out of the categorization of the Domestic Substances List.

When handled according to the label instructions, there is potential for dermal, eye and inhalation exposure for applicators, mixer/loaders, handlers and early-entry workers, with the primary source of exposure to workers being dermal. Precautionary label statements and PPE will be stipulated on product labels to minimize risk to applicators, mixers/loaders and handlers. As Botanigard 22 WP was identified as a moderate eye irritant, users will be required to wear eye goggles to minimize exposure. Also, early-entry workers will be restricted from entering areas where Botanigard ES and Botanigard 22 WP have been applied for a period of 12 hours unless wearing the indicated PPE.

The PMRA assumes that all microorganisms contain substances that can elicit positive hypersensitivity reactions, and that exposure to allergens, including *B. bassiana* strain GHA may cause allergies following repeated exposures. As a result, the signal words "POTENTIAL SENSITIZER" are required on the principal display panels of all technical and end-use products. Furthermore, appropriate PPE, including a NIOSH-approved respirator, are required on the product labels.

The label does not allow applications to turf, residential or recreational areas. As the use sites are limited to agricultural greenhouses, exposure to infants and children in school, residential and daycare facilities is likely to be minimal to non-existent. Consequently, the health risk to infants and children is expected to be negligible.

Although a secondary metabolite of *B. bassiana* strain GHA has been identified in the technical product, there was no significant toxicity and no signs of pathogenicity observed when rats were administered a batch of the technical product containing maximum levels via oral, pulmonary, or intraperitoneal routes, or when aquatic organisms were exposed in solution. The registrant proposes to monitor all future production batches to ensure that the level of this metabolite in the technical product does not exceed these levels. Calculations accounting for the maximum allowable level of this metabolite, as well as dilution rates, typical application rates, and the lack of active growth of *B. bassiana* strain GHA on the crops, indicate that maximum potential levels of beauvericin on any treated food commodity would be negligible. Therefore, the establishment of a MRL is not required for *B. bassiana* strain GHA under Section 4(d) of the *Food and Drugs Act* (adulteration of food) as defined under Division 15, Section B.15.002 of the Food and Drugs Regulations.

7.3 Environmental Risk

The information available on the environmental fate and non-target organism effects of *B. bassiana* strain GHA was determined to be sufficiently complete to permit a decision on registration.

Based on the proposed greenhouse uses of Botanigard ES and Botanigard 22 WP, the amount of *B. bassiana* strain GHA transferring to outdoor environments is expected to be low, and although *B. bassiana* strain GHA could survive in the soil under suitable environmental conditions (i.e., temperature, moisture, pH), persistence under field conditions is limited due to a rapid decrease in conidial survival when exposed to sunlight. Therefore, it is expected that the populations of *B. bassiana* strain GHA in outdoor environments would return to natural background levels over time.

The ecotoxicology data package included a field study with avian species (oral), and testing on earthworms, terrestrial plants, terrestrial arthropods (*Apthona flava*, *Xylocoris flavipes*, *Tenebrio molitor*, and *Apis mellifera)* and on parasitism between *Eretmocerus* n. sp. and whitefly (*Bemisia tabaci*) larvae, as well as a field study on the effects on various insect species. Based on the minimal exposure and weight of evidence, avian pulmonary and wild mammal toxicity/infectivity testing was not necessary. Aquatic species tested included freshwater fish, aquatic arthropods and freshwater alga. Published studies on the potential effects of *B. bassiana* strain GHA to avian species, bees and aquatic species also provided additional insight into the potential hazards to certain species.

Although the non-target insect studies did not entirely meet guideline criteria, based on the low potential outdoor exposure of non-target terrestrial organisms, and a weight of evidence from published and unpublished information, the use of Botanigard ES and Botanigard 22 WP is not expected to pose an unacceptable risk to non-target terrestrial organisms. Although the risk to the non-target insect species was found to be acceptable, in order to be protective of beneficial insects used commercially in greenhouses, the end-use product labels will state that Botanigard ES and Botanigard 22 WP may be harmful to beneficial insects and that direct contact with beneficial insects should be avoided. To further ensure that honeybees are not adversely affected, precautionary labelling will also specifically instruct users to avoid spraying when bees are actively foraging. To reduce the potential for phytotoxic effects on crop plants, advisory statements notifying users to spot test plant surfaces before applying product and to minimize the accumulation of visible residues on plant surfaces will also be required on the label under the directions for use section.

Based on results from aquatic organism testing, the low potential for exposure to aquatic environments, and a weight of evidence of the safety of *B. bassiana* strain GHA to aquatic species, it is not expected that the greenhouse use of Botanigard ES and Botanigard 22 WP will result in an unacceptable risk to non-target aquatic organisms. Standard label statements for greenhouse pest control products instruct users not to allow effluent from greenhouses to enter lakes, streams, ponds or other water bodies and are considered adequate to protect aquatic environments.

A component of one of the formulants in Botanigard ES contains aromatic petroleum distillates that are toxic to aquatic organisms and will, therefore, be labelled as such on the end-use product label.

A component of one of the formulants in Botanigard ES is considered to be toxic as defined in section 64 of the *Canadian Environmental Protection Act*, 1999. The PMRA has conducted a risk assessment of this formulant component and has found that the associated risk is acceptable for the proposed use.

7.4 Value

Botanigard ES and Botanigard 22 WP will generally provide acceptable control of whiteflies, aphids and thrips on greenhouse vegetables and ornamentals. However, depending on humidity and temperature, efficacy can be variable. Under unfavourable conditions, these products may not always provide good control.

8.0 Proposed Regulatory Decision

Health Canada's PMRA, under the authority of the *Pest Control Products Act* and Regulations, is proposing full registration for the sale and use of *Beauveria bassiana* Technical, Botanigard ES and Botanigard 22 WP, containing the technical grade active ingredient *Beauveria bassiana* strain GHA, to control whiteflies, aphids and thrips in greenhouse oranmentals and vegetables.

An evaluation of available scientific information found that, under the approved conditions of use, the product has value and does not present an unacceptable risk to human health or the environment.

List of Abbreviations

µg	micrograms
a.i.	active ingredient
ADI	acceptable daily intake
APE	alkyphenol ethylxoylate
ARfD	acute reference dose
ATCC	American Type Culture Collection
bw	body weight
cm	centimetres
CFU	colony forming units
DNA	deoxyribonucleic acid
EC_{50}	effective concentration on 50% of the population
ES	emulsifiable suspension
g	gram
ha	hectare(s)
kg	kilogram
L	litre
LC_{50}	lethal concentration 50%
LD_{50}	lethal dose 50%
mg	milligram
mL	millilitre
MAS	maximum average score
MIS	maximum irritation score
MPCA	microbial pest control agent
MRL	maximum residue limit
N	number
N/A	not applicable
NIOSH	National Institute for Occupational Safety and Health
NOEC	no observed effect concentration
PPE	personal protective equipment
PMRA	Pest Management Regulatory Agency
REI	restricted entry interval
TSMP	*Toxic Substances Management Policy*
WP	wettable powder

Appendix I Tables and Figures

Table 1 Toxicity and Infectivity of *Beauveria bassiana* strain GHA and Its Associated End-Use Products, Botanigard ES and Botanigard 22 WP

Study Type	Species, Strain, and Doses	Results	Significant Effects and Comments	Reference(s)
Acute Toxicity/Infectivity of *B. bassiana* strain GHA				
Acute oral toxicity and infectivity	Rat – CD® i. 9/sex viable MPCA in 0.1% Tween 80 at 1.05×10^8 CFU/animal (Lot # 930210GHA.A) ii. 9/sex heat-killed (HK) MPCA (1.05×10^8 CFU/animal) iii. 3/sex untreated shelf iv. 9/sex untreated Sacrifices (3/sex/group) on days 0, 3 and 7	7-day LD_{50} $> 1.0 \times 10^8$ CFU/animal (males, females)	-No mortalities, no significant toxicity, no treatment related clinical signs, no necropsy findings or changes in bw -MPCA cleared from all tissues/fluids by day 3 LOW TOXICITY, NOT PATHOGENIC	PMRA 806619
Acute pulmonary toxicity and infectivity	Rat – CD® i. 40/sex viable MPCA in 0.1% Tween 80 at 1.01×10^8 CFU/animal (Lot # 930210GHA.A) ii. 40/sex treated with heat-killed (HK) MPCA (1.01×10^8 CFU/animal) iii. 10/sex untreated shelf control iv. 40/sex untreated control Sacrifices (10/sex/group) on days 0, 3, 7 and 14	14-day LD_{50} $> 1.01 \times 10^8$ CFU/animal (males, females)	-No mortalities -Generalized inflammatory response in animals treated with viable and HK MPCA; resolved by day 14 -Females treated viable MPCA: evidence of slight systemic toxicity (temporary lack of bw gain vs. UTC); resolved by day 14 -MPCA cleared from all tissues/fluids by day 7 LOW TOXICITY, NOT PATHOGENIC	PMRA 806626

Study Type	Species, Strain, and Doses	Results	Significant Effects and Comments	Reference(s)
Intraperitoneal infectivity	Rat – CD® i. 9/sex viable MPCA in 0.1% Tween 80 at 1.05×10^7 CFU/animal (male) or 1.05×10^7 CFU/animal (female) (Lot # 930210GHA.A) ii. 9/sex heat-killed (HK) MPCA (1.01×10^7 CFU/animal) iii. 3/sex untreated shelf control iv. 9/sex untreated control Sacrifices (3/sex/group) on days 0, 3 and 7	-No mortalities and no significant toxicity -MPCA cleared from all tissues/fluids by day 3 NOT INFECTIVE, NOT PATHOGENIC	-No mortalities and no significant toxicity -MPCA cleared from all tissues/fluids by day 3 NOT INFECTIVE, NOT PATHOGENIC	PMRA 806628
Acute dermal toxicity	Rabbit – New Zealand white 5/sex at 2 g of MPCA/animal (equivalent to 1.6×10^{11} CFU/animal; Lot # 930210GHA.A) Mean dose: 0.56 g/kg bw (0.44-0.72 g/kg bw) Control groups were not used	14-day LD_{50} > 0.56 g/kg bw (males, females; mean measured)	-No mortalities or signs of overt toxicity -Dermal irritation observed in all rabbits within 1 h of removal of wrappings; persisted as red spots at day 14 (5 rabbits) Deficiencies: Dermal toxicity testing should be conducted with the EP. Dose was below recommended level of 2 g/kg bw Study was terminated before all clinical signs had cleared. ACCEPTABLE BUT SUPPLEMENTAL	PMRA 806620

Study Type	Species, Strain, and Doses	Results	Significant Effects and Comments	Reference(s)
Dermal sensitization	Buehler method Guinea pig Induction phase: topical application 1× week for 3 weeks. Challenge phase: topical application asministered 2 weeks after third induction dose For each dose phase: i. MPCA: 10 males at 0.08 g (8.2×10^9 spores) ii. Positive control: 10 males dosed with hexylcinnamal-dehyde (HCA) iii. Negative control group: 10 males untreated	The study was classified UNACCEPTABLE for the following reasons: Dose used in the induction phase was below the level required to illicit mild irritation. The test substance was not moistened during dosing.	-No mortalities occurred in any group; necropsies were not performed -No positive skin reactions observed during induction (treated or positive control group) -No positive skin reactions observed in treated or UTC groups -Positive response in 4/10 positive controls following challenge dose	PMRA 806623
Eye irritation	Rabbit – New Zealand white (6 animals) 0.1 g undiluted TGAI into the right eye (equivalent to 1.23×10^{10} conidia/eye) for 24 hrs, then flushed with 0.9% saline The left eye served as the untreated control	MIS (1 h) = 13.3 MAS = 6.8	-In two animals: corneal opacity, iridal irritation and corneal epithelial peeling was observed. -In all animals: moderate conjunctival irritation and positive irritation reactions -All treated eyes clear of positive reactions 72 hrs after treatment; eyes returned to normal appearance by day 7 MILD EYE IRRITANT	PMRA 806624

Study Type	Species, Strain, and Doses	Results	Significant Effects and Comments	Reference(s)
Genotoxicity-bacterial reverse mutation assay	Bacterial mutants were exposed to *B. bassiana* strain GHA conidia spores (TGAI) Plate-incorporation (pre-experiment) at 3, 10, 33, 100, 333, 1000, 2500, and 5000 g/plate Pre-incubation (main test) at 1, 3, 10, 33, 100, 333, 1000 and 2500 g/plate Both tests were conducted in the presence and absence of metabolic activation.	The study is classified as UNACCEPTABLE since plate test assays are inappropriate for testing microbial agents.	-Mutant strains were *Salmonella typhimurium* strains TA 1535, TA 1537, TA 98 and TA 100, and *Escherichia coli* strain WP2 uvrA -No substantial increase in revertant colony numbers in any of the bacterial mutant strains tested in either test	PMRA 1147439
Acute Toxicity/Irritation of Botanigard ES				
Acute oral toxicity	Limit test Rat – Spraque Dawley (CRL:CDRBR) 5/sex dosed orally with Mycotrol ES9601[a] at 5 g/kg bw No control groups were used	14-day oral LD50 >5 g/kg bw (male, female)	-No mortalities, no gross necropsy findings LOW TOXICITY	PMRA 806694
Dermal irritation	Rabbit – New Zealand white 5/sex dosed with 0.5 g of undiluted Mycotrol Botani Gard ES[a] for 4 hrs	MIS (1 h) = 0.333	In two animals: very slight erythema within 1 hr of exposure; persisting (1 animal) at the 24-hr scoring period -In one animal: very slight edema at the 24 hr scoring period MINIMAL SKIN IRRITANT	PMRA 806701

Study Type	Species, Strain, and Doses	Results	Significant Effects and Comments	Reference(s)
Eye irritation	Rabbit – New Zealand white 6 animals dosed with 0.1 mL undiluted ES 9601[a] into the right eye for 24 hrs, then flushed with water The left eye served as the untreated control	MIS (1 h) = 17.5 MAS = 6.1	-In two animals: corneal opacity -In four animals: iridal irritation -In one animal: corneal epithelial peeling, confirmed by a positive sodium fluorescein examination at 24 hrs. -In all animals: moderate conjunctival irritation -Prolonged pain response after treatment MILD EYE IRRITANT	PMRA 806703
Acute Toxicity/Irritation of Botanigard 22 WP				
Acute oral toxicity	Limit test Rats – Sprague Dawley (Crl:CDRBR) 5/sex dosed with Mycotrol WP9611b[b] in 50% corn oil at 5g/kg bw No control groups were used	14-day LD50 >5 g/kg bw (males, females)	-No mortalities, no gross necropsy findings, no differences in bw gain -One male: discoloration around the mouth and nose, redness around the nose fur, salivation, diarrhea, wet inguinal fur, abnormal breathing on the day of dosing; as well as rough hair coat day 4 that was resolved by day 5 -One male: discoloration around the mouth on day of dosing, as well as abnormal breathing intermittently from day 3 until the end of the study LOW TOXICITY	PMRA 806814

Study Type	Species, Strain, and Doses	Results	Significant Effects and Comments	Reference(s)
Acute oral toxicity	Limit test Rats – Sprague Dawley (Crl:CDRBR) 5/sex dosed with Mycotrol WP9611b[b] in 50% corn oil at 5g/kg bw No control groups were used	14-day LD_{50} > 5 g/kg bw (males, females)	-No mortalities, no gross necropsy findings, no differences in bw gain -In two males: redness around the nose fur on the day of dosing, resolved the following day -All rats remained normal for the duration of the study LOW TOXICITY	PMRA 806815
Acute dermal irritation	Rabbit – New Zealand white 5/sex dosed with 0.5 g of a paste of Mycotrol Botani Gard 22 WP[b] by topical dermal application for 4 hrs	MIS (1 h) = 1.833 MAS = 0.778	-In all animals: very slight to well defined erythema; persisted (four animals) through the 48-hr scoring -In three animals: very slight edema within 1 h; persisted (two animals) through the 24-hr scoring -All animals fully recovered by the 72-hr scoring MILD SKIN IRRITANT	PMRA 806821
Primary eye irritation	Rabbit – New Zealand white 6 animals dosed with 0.1 g of Mycotrol WP 9616b[b] into one eye, flushed with lukewarm water after 24 hrs	MIS (24 h) = 32.2 MAS = 18.1	-In five eyes: corneal opacity (severe in one) -In all eyes: iridal irritation and moderate to severe conjunctival irritation -In one eye: pannus formation that did not resolve until day 14 -All other symptoms in all animals had resolved by day 10 MODERATE EYE IRRITANT	PMRA 806823

bw: body weight
MAS: Maximum Average Score
MIS: Maximum Irritation Score at a given time point
[a] Mycotrol ES9601, Mycotrol Botani Gard ES : equivalent to Botanigard ES (1.21×10^{10} conidia/g)
[b] Mycotrol WP9611b: equivalent to Botanigard 22 WP (4.52×10^{10} spores/g)

Table 2 Toxicity to Non-Target Species

Organism	Exposure	Test Substance(s)	Significant Effects, Comments	Reference
Terrestrial Organisms				
Vertebrates				
Birds (American kestrel; *Falco sparverius*)	Oral (Two-part field study)	Test 1 (5 µL/g bw, single dose; N=2) i. Mycocide B GH Oil Flowable ii. Carrier control iii. Untreated control Test 2 (1 µL/g bw, single dose; N=13) i. Mycocide B GH Oil Flowable ii. Carrier control iii. Corn oil control iv. Untreated control	Test 1 No mortalities (exact duration unclear), no differences in growth rates No behavioural abnormalities Clearance and necropsies were not performed $LD_{50} > 2.5 \times 10^7$ conidia/g bw Test 2 No mortalities, no differences in movement No behavioural abnormalities No visible gross pathology upon necropsy Clearance was not assessed $LD_{50} > 5.0 \times 10^6$ conidia/g bw **LOW TOXICITY**	PMRA 847914
Birds (Japanese quail; *Cortunix coturnix Japonica*)	Oral	Published study: aqueous suspension of *B. bassiana* strain ATCC 26848 at 2.95×10^{10} spores/bird (*ad libitum*); 2/sex	There were no mortalities, no significant difference in body weight, food and water consumption, or behaviour. Tissue streaks (heart, lungs, liver, stomach and intestines) and attempts to recover *B. bassiana* from quail were negative **LOW TOXICITY, NOT INFECTIVE**	PMRA 847915

Organism	Exposure	Test Substance(s)	Significant Effects, Comments	Reference
Birds	Pulmonary/ inhalation/ injection	Waiver. Avian pulmonary toxicity and infectivity testing was not required based on the following rationale: the end-use products are intended for use in greenhouse such that the level of *B. bassiana* strain GHA in the environment will not significantly increase, and thus exposure to wild birds is minimal. Most wild birds are exposed to naturally-occurring populations of *B. bassiana* without adverse effects, and an extensive literature search yielded no reports of adverse effects on wild birds. Also, the inability of *B. bassiana* strain GHA to grow at temperatures above 35°C indicates that it will not proliferate at avian temperatures (40°C). In addition, human health data performed on laboratory animals showed no detrimental effects to mammalian species. **No additional information required.**		N/A
Wild mammals	Waiver. Wild mammal testing was not required due to the following rationale: the end-use products are intended for use in greenhouse such that the level of *B. bassiana* strain GHA in the environment will not significantly increase with the use of Botanigard ES and Botanigard 22 WP, and thus exposure to wild mammals is minimal. Most wild mammals are exposed to naturally-occurring populations of *B. bassiana* without adverse effects, and an extensive literature search yielded no reports of adverse effects on wild mammals. Also, the inability of *B. bassiana* strain GHA to grow at temperatures above 35°C indicates that it will not proliferate at mammalian body temperatures (37°C). In addition, human health data performed on laboratory animals showed no detrimental effects to mammalian species. **No additional information required.**			N/A
Invertebrates				
Non-arthropod species (Earthworm *Eisenia foetida*)	Artificial soil substrate at: 1.1×10^{10} 1.8×10^{10} 3.1×10^{10} 5.2×10^{10} 8.6×10^{10} conidia/kg soil (equivalent to 130, 216, 360, 600, or 1000 mg/kg dry soil)	i. *B. bassiana* strain GHA ii. Untreated control iii. Heat-killed (HK) control (autoclaved *B. bassiana* strain GHA at 8.6×10^{10} conidia/kg soil; 1000 mg/kg dry soil)	Mortality: 5% in 216 mg/kg treatment group; 3% in the HK control group No treatment-related effects on body weight or burrowing behaviour 14-day LC_{50} > 1000 mg/kg NOEC (body weight) = 1000 mg/kg **LOW TOXICITY**	PMRA 1147445

Vascular plants				
Vascular Plants	The potential for phytotoxicity was addressed by numerous efficacy studies conducted with Botanigard ES and Botanigard 22 WP on greenhouse ornamentals and vegetables (tomato, cucumber). Some phytotoxicity was reported on certain tomato varieties treated with Botanigard ES, as well as commercially unacceptable residues on certain plants, particularly at the higher rates. Advisory statements are required on the label under the directions for use section (i.e., spot test plant surfaces, avoid over-application). There were no reports of phytotoxicity due to *B. bassiana*, and no *Beauveria* species were identified as phytopathogens in a literature search of relevant databases. Also, the greenhouse use of Botanigard ES and Botanigard 22 WP precludes exposure to non-target plants. **No additional information required.**			PMRA 806635
Terrestrial arthropods				
Flea beetle (*Apthona flava*)	Single contact exposure: mean measured rates of 6.0×10^4 (estimate), 3.0×10^5, 7.7×10^5 and 2.2×10^6 conidia/cm^2 (equivalent to field rates of 0.62, 3.0, 7.7, and 22.0×10^{14} conidia/ha) Immediately following application, insects were placed in plastic containers (unspecified relative humidity) for observation	30/group i. *B. bassiana* strain GHA in 0.05% Tween 80 ii. Untreated control (UTC) iii. Carrier-treated group Doses were not tested for viability	Mortality in control groups was low (UTC: 7%, carrier control: 0%) Mortality in the treatment groups followed a dose-response; maximum mortality 91% on Day 10 in the 2.2×10^{14} conidia/ha group (highest rate tested) Infectivity was assessed by the frequency of sporulation of cadavers under high humidity; this is not considered a definitive assessment given that MPCA is expected on the exterior of the insect All cadavers from all viable treatment dose levels showed infectivity; no control group beetles showed infectivity 10-day $LD_{50} = 5.2 \times 10^5$ conidia/cm^2 (equivalent to 5.2×10^{13} conidia/ha)	PMRA 1474516

Warehouse Pirate bug nymphs (*Xylocoris flavipes*)	Environmental exposure (10-day, continuous) at 2.6×10^5, 2.6×10^6, and 2.7×10^7 conidia/cm^2 (nominal; equivalent to 2.6×10^{13}, 2.6×10^{14} and 2.7×10^{16} conidia/ha)	30/treatment i. *B. bassiana* strain GHA ii. Untreated control iii. Heat-killed *B. bassiana* strain GHA Viability was not assessed	A carrier control group was not used Significantly higher mortality (unequivocally attributed to MPCA exposure) observed in insects treated with viable MPCA at≥ 2.6×10^6 conidia/cm^2 compared to the attenuated control group Infectivity assessed by the frequency of sporulation of cadavers under high humidity; this is not considered a definitive assessment of infectivity given that MPCA is expected on the exterior of the insect All cadavers from all viable treatment dose levels showed infectivity The LD_{50} was not calculated	PMRA 847919

Parasitic wasp (*Eretmocerus* sp.)	Test 1. Effect on parasitism with whitefly larvae (*Bemesia argentifolii*) at 1000 conidia/mm^2 (nominal; equivalent to 1×10^{13} conidia/ha) Test 2. Effect on longevity of adult parasitoids after 3, 9, and 13 days parasitization Test 3. Effect on fecundity of mated females (after 13-day parasitization period) Test 4. Effect of direct exposure outside of host (1000 conidia/mm^2; 1×10^{13} conidia/ha)	Whitefly larvae previously parasitized for 1, 2, 3, 9 or 13 days treated with: i. Mycotrol WP, containing *B. bassiana* strain GHA, suspended in 0.01% Tween 80 ii. Carrier control group: 2nd- and 3rd- stage parasitized whitefly larvae sprayed with 0.01% Tween 80, iii. Untreated control group (UTC): parasitized whitefly larvae sprayed with water Dose measurements and viability were not supported by raw data	Test 1: Rate of successful parasitism vs. fungal infection: at Day 2, successful parasitism was significantly higher than infection; after ≥3 days, high level (≥95%) of parasitism, with a high resistance to infection (≤5%); level of successful parasitism in carrier controls and UTC not reported Test 2: Significantly reduced longevity in adults vs. carrier controls; even at 13 d of parasitization (UTC results not reported) Test 3: No effect on fecundity compared to untreated controls (results from the carrier controls not reported) Test 4: Significantly shorter longevity vs. UTC and carrier controls Infectivity was not assessed	PMRA 847921
Mealworm beetle (*Tenebrio molitor*)	Contact exposure Observed for mortality for 10 days at 27–29°C and 40–50% relative humidity	i. Low Rate: *B. bassiana* strain GHA in Mycocide OF oil carrier at 2×10^4 conidia per spray (1×10^5 conidia/mL; N=30) ii. High Rate: *B. bassiana* strain GHA in Mycocide OF oil carrier at 2.4×10^8 conidia per spray (2.6×10^9 conidia/mL, N=90) iii. Untreated control (UTC, air) iv. Carrier oil (0.2 mL, or 0.09 mL) Viability confirmed by grasshopper bioassay	Low rate: mortality in the carrier controls was the same as that with the formulated product, suggesting that the carrier oil may have insecticidal properties Infectivity was not assessed The LC_{50} was not calculated Under the conditions of the study, a considerable increase in mortality was not observed compared to untreated controls and carrier controls	PMRA 847918

Honeybee (*Apis mellifera*)	Hive study by contact exposure: 3× at 5-day intervals; returned to colonies after spraying Observation period: 30 days from last day of spraying	i. Mycotrol WP, containing *B. bassiana* strain GHA, suspended in 0.01% Silwet L77, at 2.64×10^8 spores/mL per spray (nominal; ±10%; 4.9×10^{13} spores/ha) ii. Attenuated control group: Heat-killed Mycotrol WP iii. Untreated control group (UTC) iv. Positive control: *Ascosphera apis* (agent of chalkbrood in larvae) Viability was not assessed Mean measured: 2.47×10^{13} spores/ha; 48.8% nominal. Spore counts: 2.27×10^5 spores/bee	<u>Infectivity</u> -In dead bees: incubate surface-sterilized cadavers at high humidity -In live bees: dissected sacrificed bees, plated on semi-selective media Positive controls failed to display infection; therefore control was inappropriate, or test conditions were not optimal The lack of a carrier control group was considered acceptable given the accepted safety of the carrier to honeybees. However, a reduction in exposure due to increased run-off may have been experienced, although dose/bee measurements indicate that the delivered dose was sufficiently high (con't next page)	PMRA 847917

Honeybee (*Apis mellifera*) con't	con't	con't	Worker bees: -No apparent difference in mortality between groups (visual observation of graphical data) -"Infection": proportion of dead bees positive for MPCA on water agar out of the total exposed (i.e., directly or indirectly) was ≤2.1%; no latent infections in bees sacrificed at Day 30 (plating dissected bees, semi-selective agar) An LC_{50} (worker bee survival) was not calculated (no raw data) Larval brood: -No signs of infection from two censuses -No changes in normal homeostatic processes, housekeeping, behaviour, pupal orientation, or brood nursing. Statistical analyses of the larval brood survival data were meaningless; an LC_{50} was not calculated	PMRA 847917 con't
Terrestrial arthropods: Bee (*Apis mellifera, Bombus impatiens*)	Summaries of unpublished studies: Bumblebees and honeybees treated with Botanigard ES and Botanigard 22 WP by immersion or by air-brush spraying displayed close to 50% or higher mortality upon returing to colonies, suggesting that the formulated MPCA could be toxic following direct exposure. Different strains of *B. bassiana* exhibited different virulence under laboratory conditions, with *B. bassiana* strain GHA among the higher level of mortality. Although not conclusive evidence without raw data and full methodologies, the results suggest that bees could be harmed if directly exposed under the worst-case scenario. Other studies conducted on the potential use of bumblebees as biological control vectors in greenhouses demonstrated successful delivery at effective doses without adverse effects on pollination activity or hive populations. The inoculum (Botanigard 22 WP plus corn flour) was distributed to the bees via a dispenser attached to the bee hive. Although not conclusive, the study suggests that *B. bassiana* strain GHA is innocuous to bumblebees under operational conditions of use.			PMRA 1147444 PMRA 806720 PMRA 1549765

Field study (various insects)	Field exposure: non-target insects were collected from rangeland and alfalfa fields following field application	Alfalfa site: Mycotrol ES at 3.5×10^{13} conidia/ha Rangeland: Mycotrol ES at 1.75×10^{13} conidia/ha Viability confirmed by grasshopper bioassay	Persistence in field: plate counts: MPCA counts declined logarithmically over time on vegetation samples Infectivity: plating homogenates of freeze-killed insects on semi-selective agar; raw data seldom reported Colonization by MPCA: incubating freeze-killed, surface-sterilized insects on moistened filter paper for 10 days; raw data seldom reported Grasshopper No effect on densities. Effect of environmental conditions on mycosis: MPCA-exposed grasshoppers in cages in fields, or in greenhouses showed faster development, and higher incidence of final mycoses under greenhouse conditions vs. field (Con't next page)	PMRA 847910

Field study (various insects), con't	con't	con't	Leafcutting bees (*Megachile rotundata*): Prevalence: 20% bees from hives in treated fields had 1000 CFU/bee (internal processing, 10 days post-application), with colonization and signs of infection No effect on survival of larvae or pre-pupae, or adult emergence Rangeland insects: Internal levels of the MPCA declined over 6 days postapplication; mean level on spiders, carabid and tenebrionid beetles were <25 CFU/insect; maximum level was 2×10^3 CFU/insect; No MPCA recovered from Scarabidae beetles (no raw data) No colonization in any arthropods (no raw data) Alfalfa field site: -Early post-application, internal levels significantly increased in Ladybird beetles, Harvestmen spiders, but were not considered true infection; counts in Harvestmen spiders were consistently high; levels in other arthropods were low with minor/no increases -No quantification (no raw data) Colonization in Ladybird beetles, Alfalfa weevil, Lygus bugs, and Alfalfa plant bugs	PMRA 847910 con't

Microorganisms	Waiver. The justification based on the components of the residual culture material of the TGAI being well-characterized, and monitoring of the TGAI will ensure that the level of known metabolites (i.e., beauvericin) does not exceed levels which produced no significant toxicity in mammals (oral, pulmonary, and intraperitoneal exposure), or in aquatic species (aqueous expsoure). Also, the mode of action is not known to be related to secondary metabolites, and the time course of pathogenicity and subsequent mortality are consistent with general invasive infection and not toxicity. Given the limited and indirect exposure to outdoor soil from composting greenhouse material, it is not expected that the use of Botanigard ES and Botanigard 22 WP will significantly increase the natural environmental background levels of this microorganism in the soil. Therefore, the hazard of *B. bassiana* strain GHA to non-target environmentally or economically important microbial species or microbiologically-mediated biogeochemical processes is considered to be low. **WAIVER ACCEPTED**			PMRA 806634
Aquatic Organisms				
Vertebrates				
Freshwater fish (Fathead minnow; *Pimephales promelas*)	31-day aquatic exposure (static renewal at 72-hr.) After completion of hatching (Day 4), surviving fry (20 fry/rep., 3 rep./group) transferred to retention chambers and observed for 28 days post hatch	Early life-stage: Fertilized eggs (40/rep.; 3 groups) i. *B. bassiana* strain GHA at 7.5×10^8 conidia/L (mean measured) ii. Untreated control group (UTC) iii. Attenuated control group: Heat-killed *B. bassiana* strain GHA at 7.5×10^8 conidia/L Viability in solution was confirmed (no raw data)	No effects on day-to-hatch or percent hatch of embryos Larvae survival was highest in the treated group Total length and total wet weight of the treatment group was significantly reduced at Day 31 compared to the UTC and attenuated controls, which may or may not have been attributed to the lack of adjustment of the feeding rates for the increased survival in the treatment group No signs of infection or pathogenicity from external examinations, or histopathological examination upon necropsy 31-day $LC_{50} > 7.5 \times 10^8$ conidia/L (mean measured) **LOW TOXICITY**	PMRA 847916

Invertebrates				
Aquatic Arthropods (daphnids, *Daphnia magna*)	21-day aquatic exposure (static renewal at 3× week)	20 daphnids/group (2 replicates/group; N=10) i. *B. bassiana* strain GHA at: 6.4×10^7, 1.3×10^8, 2.5×10^8, 4.7×10^8, or 9.3×10^8 (mean measured) spores/L ii. Attenuated control group: heat-killed *B. bassiana* strain GHA at 1.0×10^9 spores/L; nominal iii. Untreated control (UTC) Viability in solution was not assessed throughout the study	Infectivity was not assessed 21-day EC_{50} (survival) > 9.3×10^8 spores/L (highest concentration tested) 21-day NOEC (length)= 4.7×10^8 spores/L 21-day LOEC (length)= 9.3×10^8 spores/L	PMRA 847911
Plants				
Freshwater alga (*Selenastrum capriconutum*)	96-hour aquatic exposure (static renewal): 19, 38, 75, 150 and 300 mg/L (nominal; equivalent to 1.63×10^6, 3.26×10^6, 6.44×10^6, 1.29×10^7 and 2.58×10^7 spores/mL, respectively) Mean measured: 1.48×10^6, 2.65×10^6, 5.12×10^6, 1.46×10^7, and 2.57×10^7 spores/mL (80–113% of nominal)	1×10^4 cells/mL per test chamber; 3 rep. chambers per group i. *B. bassiana* strain GHA at one of a series of five test concentrations ii. Negative control: freshwater algal medium iii. Attenuated control: autoclaved *B. bassiana* strain GHA at 300 mg/mL	Concentrations in solution decreased over but remained 55% of nominal at test termination Viability and infectivity were not assessed 96-h EC_{50} (cell densities) = 118 mg/L 96-h EbC_{50} (area under the growth curve) = 115 mg/L 96-h ErC_{50} (growth rate) = 299 mg/L NOEC (cell density, area under the growth curve, growth rate) = 75 mg/L All calculations based on nominal values	PMRA 847912

Other		
Aquatic plants and animals	A statement from The Centre for Environments, Fisheries, and Aquaculture Science (CEFAS, United Kingdom; CEFAS 1998) claims that no members of the genus *Beauveria*, or related fungi, have any relevance in aquatic animal disease.	PMRA 1447442

References

A. List of Studies/Information Submitted by Registrant

1.0 THE ACTIVE INGREDIENT, ITS PROPERTIES AND USES

PMRA 1147406 Laverlam International Corp. 2006. Bacterial and Fungal Identifiaction in Botanigard ES formulation, DACO: M2.10.2 CBI

PMRA 1147407 Laverlam International Corp. 2006. Bacterial and Fungal Identifiaction in Botanigard WP formulation, DACO: M2.10.2 CBI

PMRA 1147408 Laverlam International Corp. 2006 Response to deficiency notes, DACO: M2.10.2

PMRA 1147409 Laverlam International Corp. 2006. Spore counts Botanigard ES formulation, DACO: M2.10.2 CBI

PMRA 1147410 Laverlam International Corp. 2006. Spore counts Botanigard ES formulation, DACO: M2.10.2 CBI

PMRA 1147411 Laverlam International Corp. 2006. Results from 5-lot analysis of TGAI, DACO: M2.10.2 CBI

PMRA 1147412 Mycotech Corp. 1991. Product Identity and disclosure of ingredients, DACO: M2.7.1 CBI

PMRA 1147413 Castrillo, L. A., J. D. Vandenderg and S. P. Wraight. 2003. Strain-specific detection of introduced Beauveria bassiana in agricultural fields by use of sequence-characterized amplified region markers, Journal of Invertebrate Pathology 82, DACO: M2.7.1

PMRA 1147414 De Hoog G. S. 1972. The Genera Beauveria Isaria Tritirachium and Acrodontium New-Genus, DACO: M2.7.1

PMRA 1147415 Mycotech Corp. 1993. SOP 03-05: Characterization of Beauveria bassiana, DACO: M2.7.1 CBI

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PMRA 1147417 Laverlam International Corp. 2006. Response to deficiency notes. The Genera Beauvaria Isaria Tritirachium and Acrodontium New-Genus, DACO: M2.7.1

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B. Additional Information Considered

i) Published Information

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